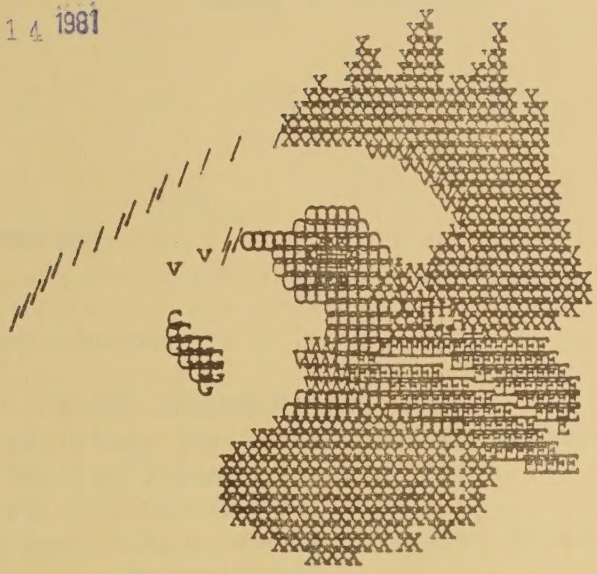

PROGRAM REVIEW OF

NRP 20450 CONTROL OF DISEASES OF POULTRY

November 12-14, 1980
East Lansing, Michigan

Science & Education Administration-Agricultural Research
U.S. Department of Agriculture

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INTRODUCTION

Several investigative groups such as the staff of the U.S. General Accounting Office and the Investigations Staff of the House Appropriations Committee have indicated that the review and evaluation of research in AR needs to be improved. From a management standpoint, it is important that administrators and staff from Washington, with the advice of action agencies, industry, and consultants, periodically reivew ongoing programs--how else can we make logical management decisions?

Program reviews are an important element in the management of research in the Science and Education Administration-Agricultural Research. Directive 600.6 entitled "Policy, Procedures, and Responsibility for Documenting and Coordinating AR Program Reviews and Workshops" establishes the AR policy that a review of the research conducted by each scientist will be performed at least once every three years. A review of research on domestic diseases of poultry was conducted in Athens, Georgia, September 22-24, 1975. Parts of the program have been reviewed since that time. However, discussions with scientists and administrators in SEA-AR led to the conclusion that the program review should be delayed until 1980. The review was held on November 12-14, 1980, at East Lansing, Michigan.

This report of the review is available to all those interested in poultry research and will be used for many different purposes. It will be distributed to all SEA-AR scientists in poultry research and to relevant administrators. Additional copies may be obtained by writing to Dr. H. G. Purchase, Acting Chief, Livestock and Veterinary Sciences Staff, Bldg. 005, Room 211, Beltsville Agricultural Research Center-West, Beltsville, Maryland 20705 or by phoning Dr. Purchase at (commercial) 301-344-3924 or (FTS) 344-3924.

OUTLINE OF PROGRAM REVIEW

1. Objectives:

   1. Review new developments in poultry disease research and examine the productivity of scientists since the last review in September 1975.

   2. Obtain an overview of current SEA-AR research effort underway (program, facilities and other resources).

   3. Examine and clarify or redefine objectives of future research in order to attack the most important poultry disease problems and develop a balanced national research program.

   4. Identify areas of cooperation between laboratories and between scientists.

   5. Make recommendations for changes in research direction or new initiatives to be undertaken.

2. Agenda

Wednesday, November 12, 1980

8:00 a.m. - Introductions.....H. G. Purchase

8:15 - Welcome.....R. L. Witter

8:30 - Objectives of Review.....H. G. Purchase

8:45 - Review of Program

(Approximate time allowed for presentation = 10 minutes + 8 minutes per SY for each area listed)

- Parasitology
- Salmonellosis
- Ornithosis
- Cholera
- Colibacillosis
- Mycoses
- Mycotoxicoses
- Mycoplasmosis
- Fowl Pox
- Adenoviruses
- Infectious Bursal Agent
- Infections Bronchitis
- Influenza
- Newcastle Disease
- Lymphoid Leukosis
- Marek's Disease
- Other Neoplasms
- Immunogenetics

4:30 p.m. - Adjourn

6:00 p.m. - Attitude Adjustment at the Witter's

7:30 p.m. - Dinner at the University Club

Thursday, November 13, 1980

8:00 a.m. - Continue Review of Programs

2:15 p.m. - Research Conducted in States.....D. D. King

3:00 p.m. - Scores Handed in for Summarization
- Tour of Laboratory Facilities

6:00 Dinner on Your Own

Friday, November 14, 1980

8:00 - 9:30 a.m. - Discussion of Importance of Programs

9:30 - 11:30 - Executive Session Involving Members of AR Review Team and Line Managers Only

11:30 - 12:00 - Feedback to Scientists and Participants, Summary and Conclusions

3. List of Participants

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MISSIONS OF POULTRY DISEASE RESEARCH LABS

Animal Parasitology Institute, Poultry Parasitic Diseases Laboratory, Beltsville, Maryland

The primary mission is the development of methods to prevent or reduce economic loss from internal parasites in poultry. This is accomplished through studies on the biology of the parasites, the pathological response of the host to infection, development of immunity, and chemical and nonchemical control methods.

Southeast Poultry Research Laboratory, Athens, Georgia

This laboratory is a center for research on diseases that cause death and condemnation losses in poultry. Basic and applied research is conducted on characterization, definition, detection, and prevention of specific diseases; influence of environment on disease development; and genetic and physiologic bases of disease control. Utilizing a high security building, the mission now also includes research to develop data for preventing, controlling, and eradicating some highly infectious and hazardous poultry diseases. Diseases being studied currently include Newcastle, avian influenza, infectious bronchitis, mycoplasmosis and salmonellosis.

South Central Poultry Research Laboratory, Mississippi State, Mississippi

The mission of this lab is to conduct fundamental and applied research on the effects of the environment, nutrition and disease on the physiology of the chicken and on poultry meat and egg production. Research on poultry diseases will concentrate on those areas where disease and environment interact.

National Animal Diseases Center, Poultry Disease Research, Ames, Iowa

The mission of this lab is to conduct basic and applied research on infectious and toxigenic diseases of poultry, particularly turkeys. Research efforts are directed to develop the techniques to prevent or control communicable diseases. Currently, research is conducted on ornithosis, colibacillosis, pasteurellosis, airsacculitis, mycoses and mycotoxicoses.

Regional Poultry Research Laboratory, East Lansing, Michigan

Researchers at this laboratory conduct basic and applied research on neoplastic and other viral diseases of poultry. The major emphasis is on developing measures and techniques for preventing, controlling, and eradicating neoplastic diseases and improving the quality, safety and consumer acceptability of poultry food products by reducing product contamination with tumor cells, viruses, and viral antigens. In the course of these studies, contributions may be made to the understanding and possible control of neoplastic disease (cancer) in other food animals and man.

Currently, research is being conducted on lymphoid leukosis, sarcomas, acute leukemia, Marek's disease, reticuloendotheliosis, squamous cell carcinoma, lymphoproliferative disease of turkeys, adenovirus infection, infectious bursal disease and immunogenetics.

REVIEW OF RESOURCES

1. Facilities and Equipment

A summary of the facililties and equipment for each lab is given in Table 1.

2. Staff and Staffing Charts

Included in the following lists are administrators who are responsible for poultry research and all scientists (SY's) contributing in part or whole to NRP 20450, Control of Poultry Diseases.

T. B. Kinney, Administrator, Science and Education Administration-Agricultural Research
- H. G. Purchase, Acting Chief, Livestock & Veterinary Sciences, National Program Staff
- D. D. King, Staff Scientist, Poultry Diseases & Production

S. C. King, Regional Administrator, Northeastern Region
P. A. Putnam, Director, Beltsville Agricultural Research Center, Beltsville, MD
H. Herlich, Chairman, Animal Parasitology Institute
- M. D. Ruff, Lab Chief, Poultry Protozoan Diseases Lab, Beltsville, MD
  Physiology and control of coccidia
- P. C. Allen, Biochemistry of coccidia
- D. R. Whitlock, Physiology and EM of coccidia
- H. D. Danforth, Immunology of coccidia

E. Kendrick, Regional Administrator, Southern Region
D. E. Zimmer, Area Director, Alabama-Georgia-South Carolina Area
W. Patterson, Assistant Area Director
- C. W. Beard, Director, Southeast Poultry Research Laboratory, Athens, GA
  Newcastle disease and influenza
- H. S. Siegel, Physiology and avian stress
- H. W. Yoder, Mycoplasma
- M. Brugh, Newcastle disease, avian influenza and inactivated vaccines
- D. J. King, Newcastle disease and infectious bronchitis
- H. D. Stone, Newcastle disease
- S. R. Hopkins, Infectious bronchitis
- J. E. Williams, Salmonella
- B. W. Mitchell, Biotelemetry, environmental engineering and computer science

- J. W. Deaton, Director, South Central Poultry Laboratory, Mississippi State, MS
  Management and disease
- T. Vardaman, Mycoplasma

P. J. Fitzgerald, Regional Administrator, North Central Region
- P. A. O'Berry, Director, National Animal Diseases Center, Ames, IA
- A. C. Pier, Chief, Bacteriology & Mycology Laboratory
  Mycoses and mycotoxicoses
- K. Rhodes, Fowl cholera and airsacculitis
- P. Rebers, " " " "
- R. Rimler, " " " "
- J. Gallagher," " " "
- K. Brogden, " " " "
- J. Richard, " " " "
- J. Thurston, " " " "
- E. McCormick," " " "

W. Mengeling, Chief, Virology Laboratory
J. Tessler, Ornithosis

N. Cheville, Chief, Pathology Laboratory
Colibacillosis
L. Arp, Colibacillosis

K. Lebsock, Director, Minnesota-Wisconsin-Michigan Area
R. L. Witter, Director, Regional Poultry Research Laboratory, E. Lansing, MI
Marek's disease
L. B. Crittenden, Lymphoid leukosis viral genetics
K. Nazerian, Marek's disease, adenoviruses and reticuloendotheliosis
J. M. Sharma, Marek's disease, immunology, infectious bursal disease, squamous cell carcinoma
L. Lee, Marek's disease, adenoviruses, immunogenetics
E. Smith, Lymphoid leukosis, Marek's disease, immunogenetics
W. Okazaki, Lymphoid leukosis
A. Fadly, Lymphoid leukosis, adenovirus infectious bursal disease, reticuloendotheliosis
L. Bacon, Immunogenetics

Distribution of Funds and Scientist Years (SY's) in SEA-AR

The distribution of funds and SY is given in Table 2.

4. Distribution of Funds and Scientist Years in the State Agricultural Experiment Stations

These data are given in Table 3.

TABLE 1. Facilities and Animal Resources for Poultry Disease Research at Different Locations - FY 1980

Laboratory	Total at Location			% used for Poultry Disease Research	Facilities for Poultry			No. Breeder Flocks			Specialized Equipment[c]
	Land (Acres)	Lab/Office ($SqFtx10^3$)	Animal Bldgs ($SqFtx10^3$)		Low[a]	Isolation Medium	High	Chicken	Turkey	Other	
NADC	400	60	20	15	0[b]	3/500	20/4 20/200	2LP	1SP	0	EMS,3EM,A,LF, DP,G,NMR,S,
PIADC	800	30	10	5	0	0	10/4 2/100	0	0	0	2EM,X,A,bLF S
RPRL	50	15	40	100	0	182/6 38/17 12/25 7/480	19/12	1 LMP 1 LP	0	Duck	1EM,A,10LF S,G
BARC-API	400	30	80	20	22/500	0	0	1	0	1	1EM,A,DP,G,LF, S,EMS
SCPRL	8	4	23	10	0	24/4	0	0	0	0	0
SEPRL	32	14	40	90	4/1000	108/4 11/500	36/4 2/240	2MP	0	0	6LF,S[d]

a. Low isolation = short term control for parasites, not for viruses.
Medium = control of introduction of viruses.
High = control of introduction and dissemination of viruses. Small units are isolators and large units are isolation rooms or FAPP buildings.

b. Numerator = number of units, Denominator = average size (sq. ft.) of each unit.

c. Abbreviations:
A = Analytical ultracentrifuge
DP = Data processing equipment
EM = Electron microscope
EMS = Scanning EM
G = Gamma counter
L = Leukosis virus free
LF = Laminar flow hoods
M = Marek's disease free
NMR = Nuclear magnetic resonance
P = PPLO (Mycoplasma) free
S Scintillation counter
X = X-ray diffraction

d. Specialized equipment is available at the Russell Research Center, adjacent to the SEPRL.

TABLE 2. Resources for Poultry Disease Research (FY 80 Proposed)

Subject Area	NADC			RPRL			BARC			SCPRL			SEPRL			PIADC		
	CT[a]	C[b]	T[c]	CT	C	T	CT	C	T	CT	C	T	CT	C	T	CT	C	T
Coccidiosis							4.1	3.6	1.5									
Histomoniasis							2.0	0.0	0.0									
Salmonellosis													1.0	2.0	0.0			
Ornithosis	2.6	0.0	1.0															
Cholera	2.1	0.0	2.7															
Colibacillosis	0.0	0.0	1.0															
Mycoses & Mycotoxicoses	0.0	0.2	1.0															
Mycoplasmosis	1.1	0.0	0.5							1.0	1.0	0.0	1.1	1.0	0.0			
Adenoviruses				0.0	0.3	0.2							0.1	0.0	0.0			
Infectious Bursal Disease				0.0	0.5	0.0												
Infectious Brochitis													1.0	1.5	0.0			
Influenza													0.1	1.0	0.5	1.0	0.0	0.0
Newcastle													3.9	3.0	0.0	1.1	0.0	0.0
Lymphoid Leukosis				4.5	3.5	0.0												
Marek's Disease				4.4	2.5	0.0												
Other Neoplasms				1.1	0.1	0.9												
Immunogenetics				0.0	1.0	0.0												
TOTAL	5.8	1.2	5.2	10.0	7.9	1.1	6.1	3.6	1.5	1.0	1.0	0.0	7.2	8.5	0.5	2.1	0.0	0.0
TOTAL SY[d]		10.6			9.0			5.1			1.0			9.8				
TOTAL $[e]		1,394			1,665			857			87			1,325				
RATIO $/SY		132			185			168			87			135				

a CT = No. of SY's on chickens and turkeys at time of previous review in 1975
b C = No. of SY's on chickens in 1980
c T = No. of SY's on turkeys in 1980
d SY = No. of SY proposed for FY 1980 from CRIS
e $ = Agency level funding proposed for FY 1980 from CRIS

TABLE 3 Distribution of Funds and Scientists in State Agricultural Experiment Stations (FY 1978)

DISEASE	RESOURCES IN SAES $(000)	SY'S
Marek's	1,337.7	10.3
Leukosis	23.4	0.1
Infectious Bursal Disease	471.2	4.6
Newcastle Disease	413.4	6.2
Influenza	223.1	5.2
Infectious Bronchitis	520.5	5.8
Adenovirus	650.2	4.1
Mycoplasma	335.4	2.6
Salmonella	290.3	3.1
Cholera	300.8	2.9
Ornithosis	25.2	0.3
Parasitology	800.1	5.7
Immunogenetics	298.2	2.6
E. Coli	259.6	1.2
Fowl Pox	37.0	0.3
Diagnostics and other	622.3	7.0
TOTAL RESOURCES	6,608.4	62.0

REVIEW OF RESEARCH PROGRAMS

1. Coccidiosis

a. Recent Progress

Certain nutritional aspects of coccidiosis have been characterized including 1) the malabsorption of essential nutrients, 2) changes in mucosal architecture, and 3) the interaction of coccidia, nutritional state of the host, and mycotoxins. Other studies have begun to identify, for the first time, the physiological and biochemical mechanisms responsible for the pathological changes and mortality induced by the infection in chickens and turkeys. Progress has been made on the *in vitro* cultivation of 3 species of turkey *Eimeria*. Studies related to the control of coccidia have shown that the 1) sporozoite invasion can be altered by modification of host cell surfaces, 2) feeding regimen affects both anticoccidial efficacy and development of immunity to coccidiosis, and 3) drug resistance may apparently be transferred during the non-sexual phase of development.

b. Objectives of Research

Identify the pathophysiological mechanisms by which coccidia affect avian hosts.

Study how current management practices, nutrition, and other disease conditions interact with coccidia.

Develop and expand the methods for control of coccidiosis, both chemical and nonchemical, with particular emphasis on immunity.

c. Research Approaches

1. Extend knowledge of how coccidia cause malabsorption of essential nutrients by the intestinal mucosa.

2. Describe the changes in organ and carcass composition resulting from coccidiosis.

3. Evaluate the relationship between coccidiosis and changes in specific plasma proteins and relate these changes to hemostasis and transport of carotenoids and vitamins in the blood system.

4. Study the biological basis for the coccidial induced interference with the metabolism of sugars.

5. Expand studies on the relationship and sequence of the events leading to death from coccidiosis.

6. Describe the influence of diet and nutritional state of the host on the course and severity of coccidial infection.

7. Measure the interaction of coccidia, with other disease conditions commonly found in the field.

8. Determine whether bird density influences coccidial severity, development of immunity and drug efficacy.

9. Characterize the effects of turkey coccidiosis on economically important parameters.

10. Determine how turkey coccidia induce the pathological changes seen and compare these mechanisms with coccidiosis in chickens.

11. Investigate the reasons for host and location specificity.

12. Examine organs, other than intestine, of chickens and turkeys for evidence of extraintestinal parasite development.

13. Define the role of phagocytic cells (macrophages) in the development of the parasite.

14. Formulate a culture medium that will support the complete development of additional species of coccidia in tissue culture.

15. Biochemically define the areas on the host cells and parasites that are involved in the invasion process.

16. Demonstrate changes in host cell surface that inhibit the invasion of the parasites in tissue culture.

17. Study the effects of immune serum on the development of each stage in the life cycle of the parasites.

18. Visualize the parasite surface antigens with ferritin and peroxidase labeling and electron microscopy.

19. Develop hybridomas that produce monoclonal antibodies against parasite antigens.

20. Develop a reproducible screening procedure for evaluating antibodies produced by the hybridomas.

21. Describe the life cycles of and develop control measures for parasites of other species of birds: pheasant, geese, ducks, quail, etc.

22. Identify the mode of action of anticoccidials.

23* Identify biological agents that destroy oocysts.

24* Develop methods to prevent or delay development of resistance to anticoccidials.

d. State of Development of the Above Approaches

1. Coccidia can cause marked malabsorption of essential nutrients depending on the species, stage, and severity of infection. Various questions about the relationship among infection, mucosal architecture, and nutrient absorption, remain unanswered including the role of compensatory absorption, contribution of mucosal changes, intestinal repair, the effects of reoccurrent

*Items added at program review

infections, the nutrient or disease state of the host, and "avirulent infections."

2-4. Significant progress is being made in identifying the mechanisms which produce the decreased weight gain, feed efficiency, and depigmentation seen with coccidiosis. Specific areas under investigation include 1) carbohydrate metabolism, 2) intestinal leakage, 3) plasma proteins, 4) carcass composition, 5) dehydration, and 6) storage of nutrient reserves.

5. Two major developments have been made in understanding the way in which Eimeria tenella induces mortality. These are 1) discovery and quantitation of a "thromboplastin-like" toxin in the cecal tissue of infected chickens, and 2) the identification of four major physiological stresses which are found only in dying birds. The nature of the toxin and the sequence of these stresses are currently unknown, nor is it known if other pathogenic species produce similar effects.

6-7. Coccidia are known to interact with Marek's disease, IBD, aflatoxin, ochratoxin, and several nutrient deficiencies. Much remains unknown about how coccidial infections are influenced by other disease conditions commonly encountered in the field and how these conditions influence 1) the progress and severity of the infection, and 2) the efficacy of control measures.

8. Current management practices are dictated by a variety of factors including economics, availability of resources, genetics, labor, consumer demand, and government regulations. These factors result in ever changing practices which can favor or hinder the development of infectious diseases. While factors such as feeding regimen, litter conditions, and medication are known to influence coccidial infections, the role of factors such as bird density, nutrient levels, drug resistance, and "development" of more virulent strains of coccidiosis are unclear.

9-10. Characterization of coccidiosis in turkeys has lagged far behind the coccidiosis in chickens. With the trend towards confinement rearing, an understanding of the physical and physiological effects of turkey coccidia has become increasingly important. Eimeria infections produce changes in organ and plasma components of young turkeys. Changes in heart size, carotenoids, vitamin A levels, and plasma protein levels are being examined in depth. Underlying causes of the changes will be examined in an attempt to explain the mechanisms producing morbidity and mortality.

11-13. Migration of Eimeria meleagrimitis sporozoites to the liver after intraperitoneal inoculation suggests the possibility of extraintestinal stages in the life cycles of some species. Tissues of infected birds other than the intestinal tissues need to be examined for the presence of the parasites. In addition, the severity of infection can influence the location of the infection. It is possible to select for strains which have a preference for specific regions of the digestive tract.

14. At present time, Eimeria tenella is the only species of Eimeria to develop through its life cycle in vitro. A chemically defined medium supported development of E. tenella and increased the early development rate of Eimeria meleagrimitis. The medium will be supplemented with metabolites known to be used by the Eimeria in an effort to obtain development of the other species. However, knowledge of the metabolic pathways used by different stages of Eimeria

is sparse, hindering efforts to develop a medium that will provide the nutritional requirements for all stages of the parasite. The need for macrophage stimulation as a requirement for development in cell cultures will be evaluated.

15-16. A procedure has been developed to assess the effects of exogenous compounds on invasion of cultured cells by sporozoites. Host cells pretreated with certain cations, enzymes, or lectins are more refractory to invasion than are untreated cells, suggesting that the treatment substances remove or change surface moities that are involved in the invasion process. Host cells and sporozoites will be treated with additional substances in an attempt to chemically define and visualize moities involved in invasion, but toxicity levels of most of the treatment compounds must first be established for cultured cells and for Eimeria sporozoites. The procedures will be modified for in vivo studies of parasite invasion.

17-18. Methods for producing immune serum for avian coccidia and for examining IFA staining of intracellular parasites have been developed. IFA staining of sporozoite surface and interior antigens have been accomplished in part, and attempts to visualize the antigenic sites with ferritin labeling and electron microscopy will be made. The effects of humoral and cell mediated responses on stages will be evaluated. The studies require the development of techniques for recovery of phagocytic cells (i.e., macrophages) from birds, and for exposing the parasites to these cells in vitro.

19-20. Techniques have been developed for obtaining large numbers of clean sporozoites for preparation of antigen. Immunized mice will provide antibody-producing splenic cells, which when fused with mouse lymphoma cells, will provide monoclonal antibody-producing hybridomas. The development of monoclonal antibodies by use of hybridoma systems requires that a reproducible screening system, adaptable to more than one class of immunoglobulin, be developed for detecting the antibodies produced in vitro.

21-22. Present approved curative and prophylactic medications are limited to use in chickens and a few in turkeys. There is a need for control measures in species of lesser economic importance which often experience severe fatal coccidiosis. In addition, the mode of action of most anticoccidials is not known.

23-24. Added at the time of the Review.

e. Problems Restricting Progress

Many of the methods to be used in the approaches are new to the field of avian coccidiosis. Techniques must be adapted from other areas to obtain the desired results. In addition, more cooperative research is needed to utilize expertise not available at the Institute. Severe limitations in financial and personnel resources are the major obstacles to the accomplishment of these objectives.

Other Parasites

Little active research is currently being conducted with poultry parasites other than coccidia. One cooperative project involving histomoniasis is nearing completion. However, the capabilities for research on other protozoa and nematodes are being retained and, in the event of disease outbreaks, studies can be resumed with little delay.

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2. Salmonellosis

a. Recent Progress

Salmonella contamination can be a problem with all meat products. This contamination may enter at any stage of that meat production, i.e. in the feed ingredients, in the mixed feed, vertical spread from parents to progeny, from infected animals to non-infected animals, from the contaminated physical environment, and because of cross contamination during slaughter and processing. Poultry meat (broilers and turkeys) are probably the most important source of salmonellae in human food.

Recent efforts indicate that formalin spray (4% and 6%) has promise as a means of decontaminating salmonella-laden poultry litter. Bacteriostatic chemicals, sulfathiazole, gentamicin, and sodium nalidixate were effective in reducing levels of salmonella infection on the gut of chickens only during and shortly after the therapy. The organisms reappeared shortly after the treatment was stopped. Salmonellae were demonstrated to survive for at least 18 months in both chicken feed and litter at 11° C.

b. Objectives of Research

Develop procedures to reduce the level of salmonella infection and resultant meat contamination in poultry.

c. Research Approaches

1. Explore the use of chemical additives to reduce the level of salmonella contamination in poultry feed.

2. Devise and evaluate means to break the vertical transmission of salmonellae from infected parents to progeny.

3. Continue efforts to develop an antigen for serogroup E without non-specific qualities to aid in serologic screening. (The other 3 antigens B, C, D, are already available).

4. Determine the relationship between the level of feed contamination and the infection rate in broilers with different Salmonellae.

5.* Role of wildlife in poultry contamination.

6.* Develop non-chemical energy efficient ways to eliminate Salmonella from poultry feeds.

7.* Devise means to eliminate Salmonella from contaminated environments.

8.* Develop integrated techniques to eliminate Salmonella from poultry flocks.

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*Items added at program review

d. Stage of development of the above approaches

1. In initial studies, one chemical shows considerable promise but it must be used in a moist to wet feed slurry, making its feasibility doubtful.

2. These studies using eggs from infected hens are underway with good transmission rates with untreated eggs. Results with treated eggs are limited and inconclusive. The work is continuing.

3. More than 100 serogroup E cultures have been examined for use as antigens. None were satisfactory because of nonspecific reactions. Efforts included using heat and chemical treatment to destory the non-specificity.

e. Problems restricting progress

The magnitude and importance of the problem necessitates that the research program be expanded. It may be feasible to produce salmonella-free broilers for the consuming public but such an accomplishment will require continuing research and a dedicated feed and poultry industry. If the industry is required to produce Salmonella-free products in the future, they will need to mount a concerted effort extending from the rendering plant to the meat counters.

f. Future plans

Continue present efforts and begin the work to relate level of feed contamination to broiler contamination.

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3. Ornithosis

a. Recent Progress

Rapid methods for serological testing and detection of chlamydial antigens in infected tissues have been developed. Preliminary results with a bacterin for protection of turkeys against virulent forms of ornithosis are encouraging.

b. Objectives of Research

Develop a rapid method for diagnosis of the presence of infection.

c. Research Approaches

1. Improve methods for rapid diagnosis by development of fluorescent antibody systems to identify chlamydia in infected tissues and tissue culture.

2. Develop different cell lines such as McCoy, BHK, PK15, and Vero cells with antimetabolites, such as cytochalasin B, cycloheximide, and cortisone acetate to detect chlamydial infected turkey tissue.

3.* Identify reservoirs of infection.

d. Stage of Development of the Above Approaches

1. The precipitin test is being used in the field for the detection of antibody. It is not suitable for identification of the organism where small numbers of organisms are present. Attempts are being made to develop the fluorescent antibody test for this purpose.

2. Try different cell lines with different antimetabolites, such as cytochalasin B, cortisone acetate, and cycloheximide to grow different strains of chlamydia.

3. Improve development of high titer antibody from turkeys and goats for fluorescent antibody work.

e. Problems Restricting Progress

1. Chlamydia is by its very nature a poor antigen as well as a labile lipoprotein.

2. The research in the tissue culture of Chlamydia psittaci has been incomplete in the United States, Canada, and Europe.

3. The antibody production in animals has not been fully understood.

f. Future Plans

It is believed that diagnosis of ornithosis will be by infected tissue surveillance of domestic flocks.

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*Items added at program review

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4. Avian Pasteurellosis (Fowl Cholera)

a. Recent Progress:

A density gradient technique was developed to purify Pasteurella multocida that were harvested from the blood of turkeys dying of experimentally induced fowl cholera. In vivo cultivated cells were more susceptible to lysis than were in vitro cultivated cells. Combination of host lysozyme, availability of divalent cations, and bacterial cell age were found to influence their susceptibility to lysis. Bacterins prepared from lysed and unlysed in vivo cultivated organisms effectively immunized turkyes against challenge with a different immunotype of P. multocida. The addition of an adjuvant to the bacterin diminished the cross-protection when it contained $\leq 5 \times 10^9$ CFU per dose but had no effect when the bacterin contained $\geq 5 \times 10^9$ CFU per dose. A technique was devised to permit in vitro cultivation of organisms without loss of the CPF. When bacteria were cultivated at 41.5°C in a medium containing turkey blood; they could be transferred 10 times without loss of the CPF. Different chemicals had the ability to modulate CPF production. It appeared that B vitamins amplified production of CPF, while amino acids were inocuous, and certain inorganic salts were repressive. The gel-diffusion-precipitin test was used to compare Westphal extracted lipopolysaccharides with the heat stable antigens presently used to serotype strains of P. multocida. The results indicated the same type specificity for the LPS as for the heat stable antigen. Although minor differences were noted, the LPS appeared to be a major component of the heat stable antigen. Protein-free lipopolysaccharide extracted from P. multocida successfully induced antibody formation in chickens. Both IgG and IgM type immunoglobulins were produced, and both were capable of passively immunizing chicks against challenge with virulent P. multocida.

b. Objectives of Research:

Develop an effective vaccine (bacterin) and improve capabilities for epidemiologic studies.

c. Research Approaches:

1. Determine bacterial and host mechanisms involved in Pasteurella multocida cross-immunity in turkeys and chickens.

2. Investigate pathogenic mechanisms of P. multocida.

3. Evaluate the relationship between chemical structure and serological specificity of P. multocida lipopolysaccharides.

4. Determine the effectiveness of P. multocida ribosomes in protecting against fowl cholera.

5. Develop serological reagents and procedures for diagnosis and quantitation of P. multocida and its antigens.

d. State of Development of Above Approaches:

1. In vivo cultivated P. multocida have been completely lysed. A soluble and insoluble fraction have been isolated and both contain CPF. SDS-polyacrylamide gel electrophoresis has shown the two fractions to be similar. The CPF in the soluble fraction is heat stable at 56°C, susceptible to proteolysis by pepsin and trypsin, and is precipitated from solution by formalin. Electron microscopic examination showed that the CPF in the insoluble fraction is associated with membraneous vesicles.

2. Numbers of P. multocida found in the blood of turkeys in the terminal stage of fowl cholera have been determined. Sterile lysates of in vivo-cultivated P. multocida equivalent in concentration to that found in those turkeys produced death and typical signs of fowl cholera.

3. Purified lipopolysaccharides have been isolated and subjected to hydrolysis to remove the lipid component. The carbohydrates of the oligosaccharide moiety are being analysed. The sequence of the carbohydrates that make up the antigenic determinants is being determined.

4. Ribosomes have been isolated from in vitro-cultivated P. multocida by two different techniques; a chemical lysis technique and a physical disruption technique. The ribosomes did not induce a detectable serological response in mice nor did they protect the mice against challenge with live P. multocida. Ribosomes induced a serological response in chickens. The ability of ribosomes to induce protection in chickens against live challenge is being tested.

5. Techniques have been developed to produce antibodies against specific capsule antigens and specific lipopolysaccharide antigens of P. multocida. Techniques have been developed which can employ avian antisera for qualitative and quantitative immunoelectrophoretic analysis of P. multocida and other antigens.

e. Future Plans:

Current research will be continued. Emphasis will be on developing a cross-immunizing bacterin and reagents to determine the immune status of vaccinated and nonvaccinated poultry flocks. Efforts will be continued to improve the serotyping system of P. multocida.

f. Problems Restricting Progress:

The major problems are the many different serotypes of P. multocida, the complexity of P. multocida antigens, and the wide distribution of the organism in domestic and wild birds.

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5. Colibacillosis

a. Recent Progress

E. coli continues to be a major cause of death and economic loss for the turkey industry. Disease may be manifest as acute septicemia, subacute fibrinopurulent serositis and airsacculitis, and granulomatous pneumonitis. Large outbreaks of colibacillosis are usually related to environmental stress or concurrent viral infections. Vaccination has not been shown to be effective and no commercial vaccine is available. Many serotypes of *E. coli* cause disease but serotype 078 appears to be most commonly involved. Virulent serotypes have been shown to be piliated while avirulent serotypes contain few surface pili. Antibiotic and sulfonamide treatments have limited effectiveness and no new progress has been made in treatment of field disease.

b. Objectives of Research

Define bacterial characteristics of virulent and avirulent strains which permit attachment and colonization of the respiratory tract and which promote entry of virulent serotypes into the bloodstream.

Isolate and purify surface pili of virulent strains and examine purified preparations for immunogenicity and as use for antigens in preparation of specific antisera.

Determine the significance of circulating antibody on opsonization and phagocytosis of *E. coli* in spleen and liver.

c. Research Approaches

1. Define bacterial characteristics of virulent and avirulent strains which permit attachment and colonization of the respiratory tract and which promote entry of virulent serotypes into the bloodstream.

2. Isolate and purify surface pili of virulent strains and examine purified preparations for immunogenicity and as use for antigens in preparation of specific antisera.

3. Determine the significance of circulating antibody on opsonization and phagocytosis of *E. coli* in spleen and liver.

4.* Develop an effective whole culture bacterin for chickens and turkeys.

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*Item added at program review

d. State of Development of Above Approaches

1. Avirulent E. coli given by aerosol shown to persist in lungs but rapidly cleared from air sac; it did not enter bloodstream or viscera. Virulent strains are cleared from lungs but rapidly penetrate bloodstream and viscera. Current studies are directed to determining efficiency of phagocytosis and intracellular destruction of avirulent and virulent strains and to why virulent strains are able to resist enzymatic destruction in phagocytic cells.

2. Methods have been devised for separation and purification of pili. Purified preparations are now being inoculated into turkeys and rabbits for further studies.

3. Passive immunization shown to produce markedly enhanced phagocytosis and killing of bacteria, even with very small amounts of antibody. Current studies are directed towards sites at which this effect occurs.

e. Problems Restricting Progress

Major problems involve (1) short duration of immunity produced by experimental vaccines, (2) multiple serotypes of E. coli that cause disease, and (3) the presence of multiple serotypes of E. coli in the intestinal tract of all turkeys. Because of these factors, eradication is not possible and vaccines are not effective. Treatment with antibiotics and sulfonamides is moderately effective in large outbreaks although annual losses remain high.

f. Future plans

Research on pathogenesis will be continued to identify virulence factors and how they operate in the turkey. Efforts will be made to produce an effective vaccine using pili preparations for use in breeder flocks.

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6. Mycotoxicoses of Poultry

a. Current Status:

Poultry species, particularly broilers and poults are susceptible to the effects of several mycotoxins, notable aflatoxin, ochratoxin, and T-2 toxin. In addition to direct losses from acute intoxication, losses also accrue from reduced growth rate, immunosuppression, and impairment of native defense mechanisms. It is believed that the latter effects, caused by levels of toxin intake below those causing overt clinical intoxication, are encountered more frequently than are acute intoxications and constitute a substantial economic loss. Poultry may be exposed to mycotoxins through contaminated feed, through toxin development in feed mixed with litter, and possibly through aerosol exposure to toxin-laden dust.

Considerable information has been developed concerning the biological effects of aflatoxin on poultry, including demonstrations of immunosuppression and impairment of resistance. There remains to be demonstrated the basic mechanisms of immunosuppression by aflatoxin. Ochratoxin has been studied fairly extensively in broilers but not in poults. There is considerable evidence to suggest that ochratoxin may effect immunosuppression through lymphoid necrosis but the subject remains to be studied. Preliminary studies indicate that T-2 toxin, and perhaps other trichothecene toxins, has immunosuppressive activity through its effect in causing thymic cortical hypoplasia and suppressing hematopoesis in some species. Vomitoxin, a trichothecene of Fusarium origin, is frequently encountered in corn in the north central United States and is prominent in the 1980 winter wheat crop in Canada; this toxin has not been studied in poultry.

b. Objectives of Research:

1. Characterize the mechanisms of immunosuppression by aflatoxin.

2. Assess the effects of aerosol exposure of poultry and rats to aflatoxin containing "dust" (i.e. toxin coated respirable particles).

3. Determine the effects of T-2 toxin and ochratoxin on cellular and humoral immune responses in poultry.

4. Determine the biological effects of ochratoxin A and vomitoxin on poults and broilers.

c. Research Approaches:

1. Define mechanisms by which aflatoxin depresses cell mediated immune responsiveness emphasizing cell-antigen interaction and lymphokine assay.

2. Assess effects of aerosolized aflatoxin on poultry and laboratory animals to determine if effective exposures are achieved by this route.

3. Assess effects of T-2 toxin and ochratoxin on immune responses of turkeys and laboratory animals.

4. Determine biological effects of ochratoxin and vomitoxin on general health of turkeys and chickens.

d. State of Development of Approaches:

(1) Aflatoxin effects on immunity: Currently experiments are being conducted in guinea pigs to determine effects on direct and indirect MIF and on direct and passively transferred delayed cutaneous hypersensitivity (DCH). Cell mediated immune reactivity, as measured by direct DCH appears to be at least equally as sensitive to aflatoxin as is weight gain response. Circulating lysozyme in turkeys does not appear to be affected by aflatoxin intake.

(2) An aflatoxin aerosol experiment has been completed in rats. Following 1 year observation period, there were no deaths but some neoplasia was noted (liver, lung, and kidney). Final interpretation of the data remains to be completed. A study using coturnix is under consideration.

(3) One study of T-2 toxin effects on immunogenesis in turkeys has been completed and published. A second study is underway which will include assessment of graft vs host reactivity.

(4) Ochratoxin is on hand for general biological effect and immunity effect assessment. Vomitoxin contaminated wheat is on request.

e. Problems Restricting Progress:

Aerosol studies require long term observation periods which make use of coturnix desirable (size and susceptibility to aflatoxin). Safety considerations with aerosolized aflatoxin must govern this approach. Mycotoxins not generally available are required that must be produced in our laboratory or sought in naturally contaminated materials. A high degree of individual variability in response to aflatoxin necessitates a large number of observations to determine results.

f. Future Plans:

Continued investigations as described on mechanisms of aflatoxin action, on cell mediated immunity, and effects of T-2 toxin and ochratoxin on immune response are planned. Studies on biological effects of vomitoxin on poultry await location of a source of vomitoxin.

7. Mycoses of Poultry

a. Current Status:

Aspergillosis continues to be a major cause of illness and death loss in the turkey industry. The disease occurs in commercial flocks raised in confinement housing. Major losses are seen in birds 14 to 22 weeks of age and in breeding toms. Changes in housing and husbandry have not controlled the disease.

Aspergillus fumigatus, the major agent of aspergillosis, has a variety of antigenic components. The antigenicity of the conidia (the infectious particle) is different than the antigenicity of the mycelium (the tissue invading form).

b. Objectives of Research:

1. Determine the pathogenesis of aspergillosis following establishment of a reproducible, safe aerosol exposure technique.

2. Develop an effective vaccine that will protect birds from environmental exposure to Aspergillus fumigatus for 22 weeks (or market age).

c. Research Approaches:

1. Studies of pathogenesis and development of an effective vaccine for aspergillosis in turkeys.

2. Develop methods to control aspergillosis spores in housing.

d. State of Development of Approaches:

Five vaccine preparations have been made (spore, mycelial, 2 germling spore and a culture filtrate). These have been tested in replicate experiments. The germling vaccine conferred considerable amounts of protection.

e. Problems Restricting Progress:

(1) The high degree of antigenic complexity of A. fumigatus including antigenic differences between the conidia (infecting particles) and the mycelia (tissue invading particles).

(2) Necessity for a reproducible closely monitored aerosol exposure challenge.

f. Future Plans:

Continued pursuit of vaccine development and use of adjuvants.

Publications

Richard, J. L., Cysewski, S. J., and Pier, A. C. Comparison of effects of dietary T-2 toxin on growth, immunogenic organs, antibody formation, and pathologic changes in turkeys and chickens. Am. J. Vet. Res. 39:1674-1679. 1978.

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Pier, A. C. An assessment of needs in mycotoxin research. Mycopathologia 65:47-49. 1978.

Pier, A. C. The effect of aflatoxin and other mycotoxins on the immune response. Proc. IV FDA Science Symp.: Inadvertant Modifications of the Immune Response FDA/OHA, 123-127, 1978.

Richard, J. L., Thurston, J. R., and Pier, A. C. Effects of mycotoxin on immunity. In: Toxins: Animal, Plant, and Microbial. Proc. 5th Intl. Symp. P. Rosenberg, ed. Pergamon Press, New York, 801-817. 1978.

Thurston, J. R., Cysewski, S. J., and Richard, J. L. Exposure of rabbits to spores of Aspergillus fumigatus or Penicillium sp: Survival of fungi and microscopic changes in the respiratory and gastrointestinal tracts. Am. J. Vet. Res. 40:1443-1449. 1979.

Richard, J. L., Thurston, J. R., and Pier, A. C. Laboratory diagnosis of mycotoxicoses of veterinary importance in the United States. Proc. 83rd Annu. Meet. U.S. Anim. Health Assoc. 205-225. 1979.

Richard, J. L. Fungi as industrial hazards. Occupational Health and Safety 43-48. July-August 1979.

Pier, A. C., Richard, J. L., and Cysewski, S. J. Implications of mycotoxins on animal disease. J. Am. Vet. Med. Assoc. 176:719-724. 1980.

Pier, A. C., Richard, J. L., and Thurston, J. R. Effects of aflatoxin on the mechanisms of immunity and native resistance. Proc. XII Intl. Cong. Microbiol. In: Medical Mycology. G. Fischer Verlag, Stuttgart, Zent. Blat. Supp. 8:301-309. 1980.

8. Turkey Airsacculitis (Mycoplasmosis)

a. Recent Progress:

Serologic studies with Mycoplasma meleagridis antigens indicated that the hemagglutination-inhibition (HI) test, commonly used to substantiate the results of agglutination tests, does not detect the same antibodies as the agglutination tests. Pathogenic synergistic relationships between avian mycoplasmas have been demonstrated. Mixed mycoplasmal infections produced more severe sinusitis and airsacculitis than infections with each of these mycoplasmas alone. A representative strain of the IJKNQR serogroup was not readily transmitted among turkeys by contact exposure, and produced little or no airsacculitis i turkeys exposed intratracheally. A simple but effective preparative procedure was developed for use in examining mycoplasmas and related organisms by scanning electron microscopy.

b. Objectives of Research:

The research objectives are to provide information on the infectious causes of airsacculitis, on improvements in methods of diagnosis, and on mechanisms by which air sac lesions are produced.

c. Research Approaches:

1. Determine mechanisms by which pathogenic mycoplasmas produce lesions in turkeys.

2. Improve reagents and procedures used for conducting hemagglutination-inhibition tests for diagnosing mycoplasmal infections in turkeys.

3. Determine the nature and significance of hemagglutinins of the pathogenic avian mycoplasmas.

4. Characterize avian mycoplasmas belonging to the IJKNQR group.

5.* Develop serological tests for the IJKNQR group of mycoplasms.

d. State of Development of Above Approaches:

1. Cell membranes isolated from Mycoplasma synoviae, strain Neb3S, and administered to turkeys by intra-air sac inoculation produced airsacculitis. The membrane preparation used as inoculum was quantitated on the basis of protein concentration. Inocula containing 5 mg of protein produced more severe air sac lesions than did those containing either 2.5 or 1.0 mg. Severity of the lesions produced was less than that of lesions resulting from experimentally induced infections with this organism. Airsacculitis resulting from membrane-inoculum containing 5 mg protein was considered moderate, while that resulting from infection was considered marked.

*Item added at program review

2. In attempts to develop more stable and convenient reagents for the HI test, formalin and gluteraldehyde fixed turkey erythrocytes have been successfully used in HI tests.

Normal turkey serums sometimes partially inhibit hemagglutination by avian mycoplasmas. Preliminary study indicated that the use of Receptor Destroying Enzyme removes this nonspecific HI effect.

Other researchers have reported the use of M. synoviae broth culture supernatant as antigen for the HI test, with the sedimented cells being used as antigen for agglutination tests. Preliminary studies with M. synoviae, M. meleagridis, and M. gallisepticum did not reveal evidence of hemagglutinating activity in broth culture supernatant; sedimented cells possessed hemagglutinating activity.

3. Procedures to solubilize and separate components of the pathogenic hemagglutinating mycoplasmas are underway. These components will be examined for biologic activity, particularly for hemagglutinating activity. The hemagglutinating mycoplasmas will be treated with substances, such as proteolytic enzymes and potassium periodate, to aid in determining the nature of the hemagglutinins.

4. Strains representing the members of the I-JKNQR serogroup have been examined with light and scanning electron microscopy. No significant differences in morphology were observed. Studies are underway to compare these strains using SDS-polyacrylamide gel electrophoresis.

e. Problems Restricting Progress:

A major problem in eliminating turkey airsacculitis is the variety of infectious agents which may be primarily or secondarily involved. Mycoplasmal infections are the most common cause of airsacculitis, and while progres has been made in attempts to develop mycoplasma-free flocks, this approach would benefit from improved diagnostic capability. Little information is available in the pathogenesis of mycoplasmal infections; this information, especially in the areas of host defense mechanisms and mechanisms by which infection produces lesions, could be helpful in developing methods to prevent disease.

f. Future Plans:

Research currently in progress will be continued. Emphasis will be on improving serodiagnostic procedures and determining mechanisms by which mycoplasmas produce lesions.

Publications

Rhoades, K. R. Inhibition of avian mycoplasmal hemagglutination by IgM type antibody. Poultry Sci. 57:608-610. 1978.

Rhoades, K. R. Comparisons of Mycoplasma meleagridis antibodies demonstrated by tube agglutination and hemagglutination-inhibition tests. Avian Dis. 22:634-638. 1978.

Gallagher, J. E., and Rhoades, K. R. Simplified preparation of mycoplasmas, an acholeplasma, and a spiroplasma for scanning electron microscopy. J. Bacteriol. 137:972-976. 1979.

Mycoplasma

a. Recent Progress

Mycoplasma gallisepticum (MG) negative flocks have been established for American broiler and turkey breeders as well as for egg production breeders. However, the spread of MG within commercial multiple-age egg laying facilities has frequently caused drops in egg production. Live MG culture exposure prior to housing pullets has been used experimentally within several states to help reduce the egg production losses. Continuous feeding of low levels of antibiotics has also been explored. The use of inactivated oil-emulsion MG vaccines is being evaluated as a possible alternative of immunization to reduce losses without further spreading live MG.

During efforts to eliminate MG from chicken breeding flocks a less virulent type of MG has been encountered in recent years. Studies indicate that these newer isolates are often less pathogenic and possibly less antigenic, giving rise to lower levels of antibodies rather than being serological variants. Similar MG infections in turkeys have also been reported recently.

The agar gel precipitin (AGP) test continues to be a rapid and specific method for serotyping most Mycoplasma isolates. In very recent studies the AGP test was used to help identify MG from naturally infected peafowl and wild turkeys. Assistance given to an APHIS epidemiological study on the incidence of Mycoplasma in numerous wild birds and animals proved that some thirty isolates of Mycoplasma encountered were neither MG nor MS. Further serotyping capabilities would be useful.

b. Objectives of Research

Develop inactivated vaccines for the control of MG infections in chickens.

Develop methods to produce and use potent MG, HA antigens in HI systems with increased stability and reproducibility.

Develop procedures for MG antibody titration in whole blood collected on filter paper strips.

Expand Mycoplasma culture typing ability to include more than just MH and MS via the AGP procedure.

c. Research Approaches

1. Develop an inactivated oil-emulsion vaccine for use in commercial multiple-age egg laying flocks to reduce drops in egg production due to the spread of MG infection.

2. Adapt the neotetrazolium blue vitally-stained MG microagglutination antigen to test whole blood samples collected and dried on filter paper strips.

3. Further establish procedures which will provide for more reproducible HA and HI titrations of antibodies when employing frozen, glycerinized MG and MS HA antigens.

4. Continue to broaden the use of the AGP procedure for the rapid and specific identification of Mycoplasma serotypes, possibly including other serotypes beyond just MG and MS.

5. Adapt the immuno-peroxidase test to rapidly identify MG and MS.

6.* Develop antigens for the identification of flocks infected with atypical or varient mycoplasmas.

7.* Generate information useful for the eradication of the new generation of mycoplasma isolates.

d. Stage of Development of the Above Approaches

1. Several formalin or beta propiolactone (BPL) inactivated MG vaccines have been produced and blended with various proportions of oil-emulsion adjuvants. These vaccines readily stimulated the production of plate agglutination and HI antibodies by the subcutaneous (SQ) route of inoculation, but somewhat lower titers via intra-tracheal (IT) route. Two vaccine doses was better than one. However, antibody titers quickly elevated following live MG challenge intranasally. One preliminary study failed to yield egg production data because pullets were housed in battery cages. The most recent study conducted in individual roll-out egg cages failed to show any protective effects from either two SQ or two IT doses of vaccine since live MG challenge did not produce a drop in egg production in even the non-vaccinated controls.

2. Several lots of neotetrazolium blue vitally-stained MG antigen have been produced which have excellent reactivity and specificity when tested with serum samples in the microagglutination system. Preliminary tests with whole blood collected and dried on filter paper strips indicate adequate sensitivity and readibility when antigen concentrations and eluted blood dilutions are serially tested.

3. Variations in HA and HI titrations from day-to-day have been reduced by (a) sonic vibration of glycerinized HA antigen batches and, by (b) the storage of that stock antigen at -60° C only after an initial further dilution of 1:100 in PBS. Some variation is still present at times.

4. Freezing and thawing Mycoplasma broth culture sediment (following centrifugation) for 10 times produces adequate AGP antigens. Adequate typing antisera can generally be prepared by intra-foot-pad inoculation of whole broth cultures of MG or MS. Similar procedures probably can be used to produce further serotype antigens and antisera.

*Item added at program review

e. Problems Restricting Progress

The major limitation of progress with current Mycoplasma research projects is that inactivated MG vaccine studies must be evaluated primarily by egg production data collected with careful accuracy and well controlled by the application of a meaningfully adequate MG challenge. Some other environmental or virus stress may be needed to make our MG challenge more realistic.

Publications

Mycoplasma

Stone, H. D., M. Brugh, S. R. Hopkins, H. W. Yoder, and C. W. Beard. Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. Avian Dis. 22:666-674, 1978.

Yoder, H. W., Jr. Serologic response of chickens vaccinated with inactivated preparations of Mycoplasma gallisepticum. Avian Dis. 23: 493-506. 1979.

10. Adenoviruses

a. Recent Progress

Although adenoviruses are ubiquitous in nature, certain strains of avian adenovirus have been shown to cause diseases in chickens and turkeys. Experimental vaccines have been used to reduce losses from such disease syndromes. Two recently isolated types of avian adenovirus are serologically and biologically distinct from all other strains. Type II adenovirus causes hemorrhagic enteritis in turkeys and marble spleen disease in chickens and pheasants; the hemagglutinating adenovirus, thought to be a duck virus, causes egg-drop syndrome in chickens.

b. Objectives of Research

To improve diagnostic methodology and study the role of the immune system in adenovirus infections at the RPRL, E. Lansing, Michigan. Maintain readiness for study of economically significant adeno-virus-induced problems which may emerge at SEPRL, Athens, Georgia.

c. Research Approaches

1. Attempt to grow type II adenovirus in cell culture systems and obtain cell-free virus for further characterization.

2. Characterize and biochemically isolate antigenic components of the hemagglutinating adenovirus.

3. Evaluate the immunodepressive capability of type I adenoviruses in chickens.

4. Develop simplified tests for reliable serological diagnosis of fowl adenoviruses.

5.* Determine the effects of type II adenoviruses on egg production.

6.* Determine the effects of type II adenoviruses on immunity.

7.* Develop a vaccine for type II adenoviruses.

d. Stage of Development of Above Approaches.

At RPRL, seed virus has been obtained. Stocks of virus and serums have been prepared.

At SEPRL, stocks of virus representing most known AV serotypes and corresponding monospecific antisera have prepared. Literature files are current. Clinical and serological responses to Adenovirus 127 have been characterized by experimental infection of commercially-reared White Leghorn and White Rock hens. Egg production and quality was decreased markedly in

*Items added at program review

virus-inoculated chickens. These effects were maximum 2-3 weeks post infection. All virus-inoculated chickens had post-infection HI $\geq$ 256, and uninoculated control chickens had titers ranging from $\leq$2 to 16. Chickens infected with a hemagglutinating adenovirus isolated from ducks by University of Georgia scientists had post-infection antibody titers to Adeno 127 virus but did not produce egg production and quality problems. These chickens were subsequently resistant to clinical effects of Adeno 127 exposure.

e. Problems Restricting Progress

Conventional serological tests for type II adenovirus are not sensitive and need to be replaced with more sensitive tests. The failures of type II adenovirus to grow in cell culture delays progress.

f. Future Plans

Continue with objectives as outlined in research approaches.

Publications

Brugh, M. and Beard, C. W. Experimental infection of commercial egg layers and broiler parents with Adenovirus 127. J. Am. Vet. Med. Assoc. 177: press, 1980

11. INFECTIOUS BURSAL DISEASE (GUMBORO)

a. Recent Progress.

Infectious bursal disease (IBD) virus causes a disease of young chickens characterized by mortality, morbidity, and immunodepression. Vaccines have been improved and consequently losses have been reduced but not eliminated. Mechanisms involved in the pathogenesis of IBD virus are not well understood.

b. Objectives of Research.

To understand the basic mechanism of host response to infection with IBD virus with emphasis on immunodepression and resistance due to age and genetic constitution and, thus, to further progress toward control of the disease.

c. Research Approaches.

1. Study the influence of IBD virus on B- and T-cell mediated immunity in chickens infected at different ages; define mechanism of immunodepression.

2. Determine the nature of age and genetic resistance to IBD.

3. Determine the role of humoral and cellular immunity in vaccinal protection against IBD.

d. Stage of Development of Above Approaches.

1. Seed virus including virulent, avirulent, and cell culture adapted strains have been obtained. Stocks of virus, antisera, and FITC-conjugated serum have also been prepared.

2. Studies have been initiated to monitor effects of IBD virus on immune competence of chickens. Preliminary indications are that this virus depresses cellular as well as humoral immune functions.

e. Problems Restricting Progress.

Although work on this research has just begun, no problems are anticipated.

f. Future Plans.

Continue as planned in research approaches.

12. Infectious Bronchitis

a. Recent Progress

Infectious bronchitis (IB) is a disease of the respiratory tract, as the name implies, but also has major impact on the poultry and egg industry as a disease of the reproductive tract. Nearly all broilers, broiler-breeders, and commercial egg flocks in the U.S.A. are vaccinated with IB virus one or more times at some stage of their grow-out. The vaccine viruses are essentially virulent viruses all capable of causing a respiratory "vaccine reaction," which is clinically indistinguishable from a natural outbreak and also causing reproductive tract disease. Vaccination programs are often designed without knowledge of the maternal antibody status of the flock, or the affect of certain routes of administration, and the vaccine is often applied concomitantly with Newcastle disease vaccine. Compounding these problems, IBV exists in a plurality of serotypes. Indeed vaccination programs in retrospect sometimes seem to have been designed to ensure not poultry health but disease stress. The fault is that insufficient research and epidemiological information is available to those responsible for the design of vaccination programs, too few diagnostic tools are available to the clinician, and too little is known about how to effectively protect respiratory tissue from disease agents in present poultry husbandry practices.

Recent advances include the description of a technique for sero diagnosis and typing of IBV, demonstration that IBV serotypes differ in their capacity to cause airsac disease in concert with Mycoplasma synoviae, and that route of administration of vaccine to pullets influences eggshell quality. High titered hemagglutination (HA) antigens for IBV are being produced consistently.

b. Objectives of Research

To improve methods to minimize losses from disease.

c. Research Approaches

1. Evaluate the role of live and killed vaccine in the control of IB.

2. Improve methods for detection and measurement of IBV and antibodies to it.

3. Define the relative capacities of various strains of IBV to precipitate airsac disease in chickens.

4. Continue work to define IBV serotypes.

5. Assemble samples of all IBV strains presently used in the manufacture of vaccine and compare serogically by SN, ELISA, and HI, pathogenicity in chickens in the absence of maternal antibody and by challenge of vaccinated chickens with selected field isolates.

d. Stage of Development of Above Approaches

Live vaccines are being studied in pullets to determine the effect of time of administration (relative to reproductive tract maturation) and route have on egg quality. Data are being gathered on antibody response to vaccination and challenge, egg production, shell quality, and strength and internal egg quality.

The conditions required for the production and storage of high titered IB-HA antigens are being defined, and the hemagglutination inhibition (HI) results obtained with these antigens compared to serum neutralization results in order to understand and interpret IB-HI results.

A few problem isolates of IBV are examined each year by plaque reduction technology to determine if a new variant or serotype has arisen in the field.

e. Problems Restricting Progress

The plurality of IBV serotypes or variants, and their undefined epidemiological significance.

The lack of understanding of the means for protecting the respiratory tract from viral infection under present intensive poultry rearing methods.

f. Future Plans

Efforts should be directed to understanding the antigenic stability of IBV (i.e., is it changing rapidly with new serotypes arising or have they long been present and unrecognized) and the epidemiological impact of these serotypes. These and present efforts would help to evaluate vaccination practices to determine if they are part of the solution or part of the problem.

PUBLICATIONS 1978-1979

Hopkins, S. R. Typing field isolates of infectious bronchitis virus by the plaque-deduction test. Avian Dis. 22:71-81, 1978.

Stone, H. D., Brugh, M., Hopkins, S. R., Yoder, H. W., and Beard, C. W. Preparation of inactivated oil-emulsion vaccines with avian viral or Mycoplasma antigens. Avian Dis. 22:666-674, 1978.

13. Avian Influenza

a. Recent Progress

Avian influenza (AI) is produced by an antigenically diverse group of Type A influenza virus and is manifest in various clinical forms that differ in severity from a fatal systemic disease to a mild respiratory tract disease. Severe forms of the disease frequently cannot be reproduced in the laboratory. Avian influenza viruses (AIV) have been isolated from commercial poultry, migratory waterfowl, imported exotic birds, and other avian species. AI is a perennial disease problem in commercial turkeys, and sporatic AI outbreaks have occurred in chickens in recent years. An agar gel precipitin test is used widely for diagnosis of AI outbreaks in commercial poultry. Vaccines have been developed to aid control of the disease.

b. Objectives of Research

1. Reduce losses from mortality and decreased egg production in turkeys.

2. Develop reliable methods for laboratory assessment of the virulence of AIV yield isolates.

c. Research Approaches

1. Assist other laboratories in identification of suspect cases of AI.

2. Develop and evaluate AI vaccines.

3. Identify biological and environmental factors which influence AIV virulence.

4.* Develop a bank of influenza isolates for vaccine antigen production.

d. Stage of Development of the Above Approaches

1. Stocks of AIV precipitin antigen and of reference antiserum are maintained for distribution upon request. Technical assistance and consultation have been provided on several occasions to diagnosticians in private and state laboratories with suspect AI cases.

2. A multivalent inactivated AIV vaccine in oil emulsion adjuvant was formulated which induced complete protection against the lethal effects of AIV challenge-exposure. This vaccine was produced commercially under contract for Minnesota and Texas turkey producers to aid in the control of recent devastating AIV outbreaks.

3. AIV (A/Ala/75) caused high mortality in a field outbreak in commercial layers, however, the disease has not been reproduced in the laboratory. Serial passage of the virus in mallard ducks did not increase its virulence for chickens in the laboratory. Also, virulence was not increased for chickens housed at environmental temperature extremes. Current efforts are directed toward determining the influence on virulence of various chemical, physical, and biological stresses.

*Item added at program review

e. Problems Restricting Progress

The multiplicity of AIV serotypes known to occur complicates control of the disease by vaccination. The lack of accurate virulence screening techniques may permit entry of highly virulent AIV strains in imported pet birds.

f. Future Plans

Research will continue at a minimal level with emphasis on approach c-3,

PUBLICATIONS

Brugh, M., Beard, C. W., and Stone, H. D. A polyvalent oil emulsion vaccine for control of influenza in chickens and turkeys. J. Am. Vet. Med. Assoc. 173 (7):886, 1978 (abstract)

Erickson, G. A., Brugh, M., and Beard, C. W. Newcastle disease and avian influenza virus stability under simulated shipping conditions. Proc. 21st Ann. Mtg. Am. Assoc. Vet. Lab. Diagnosticians (Buffalo, New York October 1978)

Brugh, M., Beard, C. W., and Stone, H. D. Immunization of chickens and turkeys against avian influenza with monovalent and polyvalent oil emulsion vaccines. Am. J. Vet. Res. 40:165-169, 1979.

Brugh, M., and Beard, C. W. Collection and processing of blood samples dried on filter paper for microassay of Newcastle disease virus and avian influenza virus antibodies. Am. J. Vet. Res. 41 (9):1495-1498, 1980.

a. Recent Progress

Veterinary regulatory authorities continue to detect sporadic outbreaks of viscerotropic velogenic Newcastle disease (VVND) in pet birds. These outbreaks until now have been successfully eradicated, but introduction of VVND virus (VVNDV) into commercial poultry could result in economically devastating disease losses. The poultry industry is also confronted with less severe disease problems which may be caused by the less virulent endemic strains of Newcastle disease virus (NDV). Live virus or inactivated virus vaccines, or both, can induce protection against the disease but not against the virus infection. Virus isolation techniques and techniques for serological assessment of flock immune status have been improved. Routine assessment of flock immune status is essential for optimal use of live virus vaccines

b. Objectives of Research

1. Prevent introduction of VVND in United States poultry flocks.

2. Minimize the economic impact of VVND should it gain entry.

3. Reduce economic losses associated with endemic strains of NDV.

c. Research Approaches

1. Develop and evaluate vaccines and vaccination programs for control of Newcastle disease.

2. Develop improved methods for assessing poultry flock immune status.

3. Determine epidemiological significance of subclinical VVND virus infections in commercial poultry and pet birds.

4. Develop information and techniques required for implementing sound VVND regulatory policy.

5.* Develop a rapid *in vitro* method for identifying VVND.

d. Stage or Development of the Above Approaches

1. Oil emulsion adjuvants are more potent immunostimulators than aluminum hydroxide adjuvants in inactivated NDV vaccines. The factors which influence potency of oil emulsion vaccines are not clearly defined but appear to relate to emulsion composition and antigen mass. Efforts are currently directed toward maximizing the potency of inactivated vaccines by manipulating the adjuvant composition so that antigen mass requirements, and thus production costs, can be minimized.

2. Live virus vaccines induce higher levels of protection against VVNDV infection following challenge-exposure than do inactivated virus vaccines. If used properly, both types of vaccine induce complete protection against clinical manifestation of disease. The ultimate objective of vaccination program development and evaluation efforts is to identify a program which will induce solid and lasting protection against infection by VVNDV.

*Item added at program review

3. Development of a simple and economical bird blood processing technique now permits flock attendants to collect blood samples on paper and mail them to the laboratory for serological assessment of flock immune status without compromising flock security. Studies of the source of variation in Newcastle serological (HI) test results suggest that the variation, both between and within laboratories, can be minimized by carefully defined and standardized test procedures. Further simplified serological monitoring techniques may be desirable.

4. Reliable methods of detecting subclinical or inapparent VVNDV infections are required for accurate assessment of epidemiological significance of the infections. Continuous administration of inactivated NDV antigen in drinking water does not enhance virus isolation rates from VVND-convalescent chickens Virus recovery is only slightly increased by tryptic digestion of samples to reactivate virus neutralized by antibody. Although nondestructive methods of sampling are the ultimate aim, current studies are directed toward assessment of tracheal-ring organ culture methods for detection of long-term VVNDV shedding.

5. Logistic constraints associated with the use of embryonated eggs for isolation of VVND virus can be relieved in some instances by use of fragments of chorioallantois cut from embryonated eggs, thereby permitting assay of about 10 samples in fragments from one egg. A commercially available bacteriological medium appears to be an excellent stabilizer for VVND virus during transport to diagnostic laboratories. Gel-packs are more suitable than dry ice for refrigeration of samples during transport. Current studies are directed toward determining the persistence of VVNDV in carcasses and feces of infected birds.

6. The effect of environment conditions on the severity of NDV infections is not clear. Current studies involve determination of environmental temperature effects on virus susceptibility, shedding, and airborne transmission.

e. Problems Restricting Progress

Successful eradication of VVND from commercial poultry, should it be reintroduced, will be contingent upon early detection to prevent widespread dissemination. Vaccination to prevent Newcastle disease problems associated with endemic strains of NDV may mask clinical VVND signs and make early VVND detection difficult.

f. Future Plans

Continue approaches outlined above.

PUBLICATIONS

Newcastle

Brugh, M. A simple method for recording and analyzing serological data. Avian Dis. 22 (2): 362-365, 1978.

Brugh, M. Butylated hydroxytoluene: A possible cause of Newcastle disease vaccination failures. Proceedings XVI World's Poultry Congress (Rio de Janeiro, Brazil, September, 1978). Vol. 6:907-916.

Brugh, M. The antiviral activity of butylated hydroxtoluene and related compounds. J. Am. Oil Chem. Soc. 55(3):248A, 1978 (abstract)

Brugh, M., Beard, C. W., and Wilkes, W. J. The influence of test conditions on Newcastle disease hemagglutination-inhibition titers. Avian Dis. 22(2): 320-328, 1978.

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Erickson, G. A., M. Brugh, and C. W. Beard. Viscerotropic velogenic Newcastle disease in domestic pigeons. Clinical disease and immunization. J. Am. Vet. Med. Assoc. 175 (6) (1979):618 (Abstract)

Brugh, M., and Beard, C. W. Collection and processing of blood samples dried on filter paper for microassay of Newcastle disease virus and avian influenza virus antibodies. Am. J. Vet. Res. 41(9):1495-1498, 1980

Brugh, M., Erickson, G. A., and Beard, C. W. Embryonated eggs compared with fragments of chorioallantois attached to egg shell for isolation of Newcastle disease virus. Avian Dis. 24 (2):486-492, 1980.

Erickson, G. A., Brugh, M., and Beard, C. W. Viscerotropic velogenic Newcastle disease in pigeons: Clinical disease and immunization. Avian Dis. 24 (1): 257-267,1980.

Stone, H. D., Brugh, M., Erickson, G. A., and Beard, C. W. Evaluation of inactivated Newcastle disease oil emulsion vaccines. Avian Dis. 24 (1): 99-111. 1980.

15. LYMPHOID LEUKOSIS

a. Recent Progress.

The rate of congenital transmission of lymphoid leukosis (LL) virus varies from less than 1% to over 50% in commercial chickens. It is now recognized that LL virus infection can reduce productivity, livability and egg size as well as induce LL, usually at low rates, in adult chickens. Assays for LL virus infection and congenital transmission have been improved, simplified and applied by commercial breeders to reduce congenital transmission. Model trials have eradicated infection in one generation, identified sources of hatchery cross infection and identified minimum isolation procedures for preventing horizontal infection while rearing small groups until infected groups are identified. Twelve structural genetic loci controlling endogenous lymphoid leukosis virus expression have been identified. Some of these loci have been shown to affect the response to exogenous LL virus infection. The LL bursal target cell has been implicated in genetic resistance and tumor latency suggesting that the immunity to tumor antigens or other systemic host factors are less important in resistance than previously thought. The region of the viral genome important for LL induction has been identified and probably these viral genes induce LL by activation of normally latent host genes.

b. Objectives of Research.

Reduce economic losses due to LL virus infection.

Improve product quality by reducing virus, viral product and tumor cell contamination.

c. Research Approaches.

1. Develop improved assay methods for LL virus to facilitate eradication and reduction programs.

2. Evaluate economic impact of exogenous LL virus infection on layer and broiler flocks.

3. Provide industry with practical programs for reduction and eradication of exogenous LL virus and assist in their implementation.

4. Determine factors that influence rate of congenital LL transmission and resistance to LL tumor development using immunological and molecular methods.

5. Identify viral genes for LL tumor induction in order to develop apathogenic strains for possible immunization to prevent virus infection or transmission.

6. Study the inheritance of endogenous leukosis virus expression and the effect of virus expression on pathology and productivity.

7. Investigate methods for germline integration of new genes for resistance to LL virus infection and other economically desirable traits.

8. Effect of lymphoid leukosis virus infection on efficiency of genetic selection.

d. Stage of Development of Approaches.

1. Direct assays for LL virus antigen in albumens or cloacal swabs using complement fixation or enzyme immunoassays have been developed and are in use for identifying shedding chickens. Enzyme immunoassays for antibodies are being developed. Work on assay methodology will be continued as needed for LL virus reduction and eradication studies.

2. Reduced LL virus infection rate has been associated with improved egg production and related traits as well as lower tumor mortality. Plans are being developed to further assess the economic impact of infection, particularly on broiler growth and feed conversion.

3. Using assay methods developed at this laboratory, several egg production breeders have substantially reduced congenital infection with LL virus by breeding from dams that do not shed viral antigen in egg albumen. About 20% of flocks do not respond to this selection. We are conducting experiments to determine the reason for failure in some flocks and plan to cooperate with breeders in attempting to reduce shedding in genetically superior flocks that are difficult to select for reduced vertical transmission. Practical methods for small group isolation of progeny while selecting groups free of infection for eradication programs have been developed so that breeders who have successfully reduced infection rate will have practical methods for complete eradication.

4. Genetic and age resistance to LL tumor development appear to be dependent on the bursal target cell and we have not been able to identify a major influence of the immune system on resistance. Molecular studies suggest that LL tumors are initiated by activation of normal host genes. Therefore, we do not think stimulation of anti-tumor immunity by a vaccine is a likely approach to control. Future studies will be largely aimed at understanding host factors controlling immune tolerance and congenital transmission.

5. We have been unable to locate or produce by biological methods apathogenic exogenous LL virus stocks. Endogenous leukosis viruses have little or no oncogenic potential but antibodies to them do not neutralize exogenous viruses. Viruses that are recombinants between exogenous and endogenous leukosis viruses have the antigenicity of endogenous viruses, but induce LL at a high rate. These studies have localized the genes for tumor induction in the viral genome. Exogenous and endogenous viral DNA has been cloned in bacteria and successfully transfected to cell cultures. Collaborative work will be aimed at using molecular methods to develop virus strains with antigenicity of exogenous viruses that are apathogenic. These viruses could serve to boost anti-viral immunity and restrict shedding in reduction programs.

6. Eight single-dominant structural genes for endogenous leukosis virus expression have been identified in our inbred lines. Congenic lines for each are under development. A line free of endogenous viral genes has been developed. Further studies of inheritance will not be initiated. The congenic lines will be used to study the effect of these genes on development of neoplasms directly or in conjunction with other oncogenic agents. The effect of these genes on immune response and reproductive traits will be studied to determine if breeders should be concerned with them in selection programs.

7. LL virus genes have been cloned in bacteria and collaborators are attempting to produce clones of DNA that have genes that code for exogenous viral antigens, and are active when inserted in somatic cell DNA. We plan to develop methods for inserting genes in the chicken germline using such DNA. If successful, we would insert a dominant gene for resistance to LL virus infection into chickens. This approach is intended as a model for inserting beneficial genes in the germlines of domestic animals.

e. Problems Restricting Progress.

The fact that some genetically desirable lines do not respond to selection for reduced LL virus transmission reduces the ability of some breeders to produce high quality low transmitting lines. Lack of agreement among breeders on the importance of LL virus reduction slows overall industry progress toward this goal.

f. Future Plans.

We will follow the approaches outlined in Section c.

Publications for the Calendar Years 1978 and 1979

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9. Neumann, U. and Witter, R. L. Differential diagnosis of lymphoid leukosis and Marek's disease by tumor-associated criteria. II. Studies on field cases. Avian Diseases 23:426-433, 1979.

10. Fadly, A. M. and Bacon, L. D. Bursal and thymic lesions in chickens bearing progressive Rous sarcomas. Avian Diseases 23:529-533, 1979.

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15. Crittenden, L. B., Witter, R. L., and Fadly, A. M. Low incidence of lymphoid tumors in chickens continuously producing endogenous virus. Avian Diseases 23:646-653, 1979.

16. Okazaki, W., Burmester, B. R., Fadly, A., and Chase, W. B. An evaluation of methods for eradication of avian leukosis virus from a commercial breeder flock. Avian Diseases 23:688-697, 1979.

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16. MAREK'S DISEASE

a. Recent Progress.

Widespread use of the herpesvirus of turkeys (HVT) vaccine has maintained Marek's disease (MD) losses at relatively low levels. However, reports of sporadic vaccine breaks continue to surface and substantial research has been directed towards understanding vaccinal immunity and minimizing vaccine breaks. Research technology in MD has reached a high level of sophistication and application of new immunologic and molecular techniques has substantially enhanced the understanding of this disease.

b. Objectives of Research.

Improve MD control by acquiring knowledge of resistance mechanisms and utilizing this knowledge to improve vaccine protection and reduce vaccine breaks.

Improve product quality by reducing virus, viral product and tumor cell contamination.

c. Research Approaches.

1. Determine the role of variant MD virus biotypes in vaccine breaks.

2. Select improved MD-type, HVT, or polyvalent vaccines for use in breeders (alternate generation system) or in commercial progeny.

3. Improve vaccine efficacy by delaying exposure through hatchery holding or by restricting infection through augmentation of MD-specific maternal antibody.

4. Study natural immune mechanisms such as the natural killer cell activity in chickens and determine their role in genetic and vaccine-induced resistance to MD. If natural immunity is important, then find ways to enhance expression of such immunity to improve resistance to MD.

5. Evaluate cellular and humoral immunity in chickens with MD and study the role of immunocompetent cells that infiltrate tumors. Develop necessary tests to evaluate immunity.

6. Develop and use hybridoma technology to prepare monoclonal antibodies against the MD tumor-associated surface antigen (MATSA) and utilize this antibody in pathogenesis studies and differential diagnosis.

7. Study the mechanism of cellular transformation. Develop an *in vitro* assay for transformation of lymphoid cells by MD virus and determine possible role of tumor promoters in tumorigenesis by MD virus.

d. Stages of Development of the Above Approaches.

1. Variant biotypes of MD virus such as the Md/5 isolant have been obtained and characterized. About 40 new isolants from MD break flocks or normal control flocks are being characterized for pathogenicity in vaccinated chickens.

2. Preliminary evaluation of attenuated Md/11 (a variant biotype) and SB-1 vaccines has been accomplished. Several newly isolated MD viruses appear avirulent and will be compared to SB-1. Polyvalent vaccines are also under study.

3. Experiments on exposure delay through hatchery holding or on breeder immunization to boost Marek's disease-specific maternal antibody have recently been initiated.

4. Natural killer cell activity has been detected in normal chickens. The role of natural killer cells in pathogenesis of MD and in natural and vaccinal resistance is being investigated. Preliminary results show that after MD virus infection, natural killer cell activity is enhanced in resistant chickens but is decreased in susceptible chickens.

5. Several cellular immune responses against viral and tumor antigens have been studied by such in vitro tests as the lymphocyte cytotoxicity assay and mixed lymphocyte reaction. The role these responses play in pathogenesis and resistance is being actively researched. The same tests also revealed that, in addition to the transformed cells, MD tumors also contain a large proportion of immunocompetent cells. The role of these cells in tumorigenesis will be determined.

6. Development of hybridoma technology is at a preliminary stage.

7. Many lymphoblastoid cell lines and transplantable tumors have been developed from MD tumors. Virological and antigenic properties of these lines have been determined. In vitro transformation of lymphoid cells by MD virus and relationship of this transformation with tumor specific antigen and the effect of tumor promoters and carcinogens on oncogenesis of MD virus need to be studied.

e. Problems Restricting Progress.

The mechanism of vaccine protection seems complex and no satisfactory in vitro correlates of vaccinal resistance exist. Diagnostic laboratories cannot reliably differentiate Marek's disease from lymphoid leukosis.

f. Future Plans.

Continue work on the research approaches outlined in Section c, above.

Publications for the Calendar Years 1978 and 1979

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2. Lee, Lucy F., Nazerian, K., Witter, R. L., Leinbach, Susan S., and Boezi, John A. A phosphonoacetate-resistant mutant of herpesvirus of turkeys. Journal of the National Cancer Institute 60:1141-1146, 1978.

3. Lee, Lucy F., Sharma, Jagdev M., Nazerian, Keyvan, and Witter, R. L. Suppression of mitogen-induced proliferation of normal spleen cells by macrophages from chickens inoculated with Marek's disease virus. Journal of Immunology 120:1154-1159, 1978.

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8. Nazerian, K., Neiman, P., Okazaki, W., Smith, E. J., and Crittenden, L. B. Status of endogenous avian RNA tumor virus in Marek's disease lymphoblastoid cell lines and susceptibility of those lines to exogenous RNA tumor viruses. Avian Diseases 22:732-741, 1978.

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12. Neumann, U. and Witter, R. L. Differential diagnosis of lymphoid leukosis and Marek's disease by tumor-associated criteria. I. Studies on experimentally infected chickens. Avian Diseases 23:417-425, 1979.

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14. Sharma, J. M. Immunosuppressive effects of lymphoproliferative neoplasms of chickens. Avian Diseases 23:315-327, 1979.

15. Sharma, J. M. and Coulson, B. D. Presence of natural killer cells in specific-pathogen-free chickens. Journal of the National Cancer Institute 63:527-531, 1979.

16. Lee, L. F., Nazerian, K., Witter, R. L., Leinbach, S. S., and Boezi, J. A. Altered biological and biochemical properties of a phosphonoacetate-resistant mutant of herpesvirus of turkeys. Oncogenesis and Herpesviruses III/Part I, pp. 253-260, 1979.

17. Kaschka-Dierich, Christine, Nazerian, Keyvan, and Thomssen, Reiner. Intracellular state of Marek's disease virus DNA in two tumour-derived chicken cell lines. Journal of General Virology 44:271-280, 1979.

18. Witter, R. L., Lee, Lucy F., Bacon, L. D., Smith, E. J. Depression of vaccinal immunity to Marek's disease by infection with reticuloendotheliosis virus. Infection and Immunity 26:90-98, 1979.

19. Lee, Lucy F. Macrophage restriction of Marek's disease virus replication and lymphoma cell proliferation. Journal of Immunology 123: 1088-1091, 1979.

20. Sharma, J. M., Herberman, R. B., Djeu, Julie Y., and Nunn, Myrthel E. Production of migration inhibition factor by spleen cells of normal rats upon culture in vitro with tumor cells and cells expressing endogenous virus. Journal of Immunology 123:222-231, 1979.

21. Nazerian, K. Marek's disease lymphoma of chicken and its causative herpesvirus. Biochemica et Biophysica Acta 560:375-395, 1979.

17. OTHER NEOPLASMS

a. Recent Progress.

Although lymphoid leukosis (LL) and Marek's disease (MD) are common diseases, other neoplasms including reticuloendotheliosis (RE), lymphoproliferative disease (LPD), and squamous cell carcinoma are of emerging or potential importance. Infection with RE virus is not presently recognized as economically important in the United States, although losses associated with RE virus-contaminated biologics in chickens or natural RE virus infection of turkeys have been noted in other countries. Defective and nondefective types of RE virus are now recognized and have been extensively characterized at the molecular level. Nondefective RE virus induces lymphomas in chickens indistinguishable by standard pathologic criteria from lymphoid leukosis and some strains depress cellular and humoral immunity. Squamous cell carcinoma is responsible for an increasing and often significant level of condemnations in broilers. LPD of turkeys, caused by a retrovirus, has not yet been reported in the United States, but causes significant losses in Europe and the Middle East. Other neoplastic diseases occur but are not of major importance at present.

b. Objectives of Research.

Develop diagnostic methodology, monitor prevalance, and design control programs for other neoplastic diseases of potential economic importance in poultry.

c. Research Approaches.

1. Determine through serologic surveys the prevalence and economic importance of RE virus infection in poultry flocks and biologics; develop improved assays.

2. Adapt molecular hybridization technology to the differentiation of lymphoid neoplasms caused by RE, LL, LPD and MD viruses.

3. Characterize the development and nature of RE virus-induced lymphomas through immunological and molecular studies and relate findings to oncogenesis of LL.

4. Determine by animal inoculation or human seroepidemiological studies whether RE viruses constitute a hazard to human health.

5. Determine the transmissibility of squamous cell carcinoma and whether the tumors are caused by an infectious agent.

6. Maintain a diagnostic and research capability for LPD of turkeys and other neoplastic diseases of potential economic importance.

d. Stage of Development

1. Over 3,000 sera have been collected by APHIS and RPRL from primary chicken breeder flocks, commercial broiler and layers, backyard

chickens and turkeys. Thusfar, none of over 1,000 sera from primary chicken breeder flocks contained FA antibody but several turkey flocks were positive. Assay of the remaining samples for SN and AGP antibodies is being initiated. A sensitive CF test (COFAR) for RE virus antigens has been developed. Elisa and SN tests for antibody detection are under development.

2. Molecular probes for RE virus and LL virus have been prepared and show distinctive tumor-specific hybridization patterns with DNA extracted from respective RE and LL lymphomas. Probes for MD and LPD are to be developed. The value of the probes in tumor diagnosis will be tested.

3. Lymphomas similar to LL have been induced by nondefective RE virus strains. A cell line has been produced from a lymphoma induced by RE virus strain CS. Preliminary studies suggest the RE lymphoma is an immunoglobulin-producing, B-cell tumor. Tumor induction is greatly reduced by bursectomy. Collaborative studies on the mechanism of RE lymphoma induction by molecular techniques are in progress.

4. RE viruses grow in dog thymus cells and may have been originally derived from mouse viruses. This approach is in the planning stage, no studies have been initiated.

5. Live chickens bearing squamous cell carcinomas are being sought as starting material for transmission studies.

6. A file of pertinent literature on LPD of turkeys is maintained. Liaison with laboratories in England and Israel is active. An Israeli project to assess the incidence of LPD and RE virus in California turkeys will be monitored. If the disease is suspected or recognized, studies on its prevelance will be initiated.

e. Problems Restricting Progress

A convenient and sensitive assay for RE virus antibodies is not yet available; AGP or FA antibodies do not persist for long periods. The availability of fresh material from cases of squamous cell carcinoma or turkey leukosis is limited at the present time.

f. Future Plans

Continue approaches outlined above.

Publications for the Calendar Years 1978 and 1979

1. Witter, R. L. and Crittenden, L. B. Lymphomas resembling lymphoid leukosis in chickens inoculated with reticuloendotheliosis virus. International Journal of Cancer 23:673-678, 1979.

2. Witter, R. L., Lee, Lucy F., Bacon, L. D., Smith, E. J. Depression of vaccinal immunity to Marek's disease by infection with reticuloendotheliosis virus. Infection and Immunity 26:90-98, 1979.

18. IMMUNOGENETICS

a. Recent Progress

Alleles at the B blood-group locus and/or closely linked loci in the major histocompatibility complex are known to influence Marek's disease (MD) resistance and the B^2 gene in $[(6_3 \times 15_1)F_{4-5}]$ chickens determines greater resistance to MD than B^5, determines fuller regression of Rous sarcomas, and affects the type and frequency of lymphoid leukosis (LL) tumors in chickens. Seven different B-alleles of variable tumor regression potential are being introduced into one susceptible inbred line to study the mechanism that purportedly leads to general tumor resistance, and to relate this resistance to other poultry diseases. The Th-1 locus determines antigens on thymus lymphocytes and has influenced LL mortality in one experiment. Blood-group antigens have detected breeding errors in 3 of 10 inbred chicken lines at the RPRL, and have permitted purification of the lines by discarding unpure families.

b. Objectives of Research.

Improve the disease resistance of chickens by (1) identifying genes at alloantigen-determining loci that influence a general resistance to viral induced neoplasms, and then establish the mechanism of the resistance and whether the resistance is directed to other diseases, and (2) developing practical tests of immunological competence that will correlate with, and allow for selection of, optimal cellular and humoral immunity to any disease organism.

c. Research Approaches.

1. Introduce several B-alleles characterized for MD and Rous sarcoma resistance into a susceptible inbred line for development of B-congenic strains to determine the dominance of the B-influence in very susceptible strains, to further characterize its general effectiveness for different neoplastic diseases, and to characterize the mechanism of action.

2. Study the influence of the IgG-1 and Bu-1 loci which determine antigens on bursal lymphocytes, and of the Th-1 and Ly-4 loci which determine antigens on thymus lymphocytes, upon MD, Rous sarcoma and LL.

3. Determine if ontogeny of some in vitro immune response(s), for example, mixed lymphocyte response and/or titre of antibody produced to a standard bacteria, is correlated with a relative ability to develop an adequate immunity following infection with poultry pathogens.

d. State of Development of Above Approaches

1. Seven B-alleles have been introduced into inbred line $15I_5$ chickens that are highly susceptible to MD and LL. The 4th backcross (BC) generation will be produced this winter. The partially congenic lines will determine dominance and generality of resistance to different tumors or diseases, but more fully congenic birds derived after additional BC will be desirable to establish the mechanism. A mouse colony has been established and are developing specific hybridoma antibodies to each of the 7 B alleles in the congenic strains.

2. The Th-1 locus has had a weak but significant influence on Rous sarcoma progression and has significantly influenced LL but did not influence MD. We have breeders to further study the effect on MD in [(6x15)F_6] chickens. The Ly-4 locus has not influenced Rous sarcomas, but did influence MD; this will be checked with [(6x15)F_6] birds. The influence of an Ig allotype locus or the Bu-1 locus on tumor resistance has not yet been studied. F_2 chickens of inbred lines resistant or susceptible to MD and LL have been produced and identified for genes at the IgG-1, Bu-1, Th-1 and Ly-4 loci. F_3 chicks of the different parental IgG-1, or Bu-1, types will be studied for resistance to LL, Rous sarcoma and MD.

3. The mixed lymphocyte response (MLR) is an in vitro cell mediated blastogenic correlate of graft-vs.-host response (GVHR). Magnitude of GVHR has been associated with B-genotype and therefore we have been developing the MLR test to see if difference in ontogeny of MLR competence is correlated with B-genotype. Inbred strains characterized for MD resistance do differ in ontogeny of MLR competence, and surprisingly, the age of the stimulator cells is very critical for MLR proliferation.

e. Problems Restricting Progress

1. Complexity of B-locus - but there is generally strong linkage disequilibrium.

2. Time of development of congenic lines - limited to 2 generations per year. One need to establish effect first.

f. Future Plans

1. Use congenic lines for other diseases.

2. Use B-antisera that detect beneficial or harmful B-antigenic determinants to screen problem commercial lines.

3. Resolve if the genotypes desirable for cell mediated immunity are those desirable for humoral immunity.

Publications for the Calendar Years 1978 and 1979

1. Lee, Lucy F. Chicken lymphocyte stimulation by mitogens: A microassay with whole-blood cultures. Avian Diseases 22:296-307, 1978.

RELATIVE IMPORTANCE OF RESEARCH AREAS

1. Research approaches

Immediately after each presentation, there was a discussion. During the discussion, additional research approaches were proposed. (The research approaches are listed under the REVIEW OF RESEARCH PROGRAMS). Then all participants scored all research approaches as follows:

Score	Interpretation
1	Highest priority, additional $ and SY, needs increased emphasis
3	Moderate priority, continue as is, okay at current level
5	Lowest priority, discontinue project, do not begin project

According to these criteria, a score of 3 is a neutral score and indicates no recommendation for change. The scores are summarized in Table 4.

The grand average score indicates that the following are highest priority or need increased emphasis: monoclonal antibody preparation for coccidiosis and Marek's disease; development of a pili or whole culture vaccine against colibacillosis; an inactivated vaccine for mycoplasmosis; attempts to grow type II adenovirus in cell culture; improved methods of detection and measurement of antibody to infectious brochitis; determine biological and environmental factors which influence influenza virulence; develop a bank of isolates of influenza for vaccine antigen production; and develop rapid in vitro methods to identify pathogenic Newcastle disease. Areas which are of lowest priority or warrant decreased emphasis include, amongst others, studies of the action of anticoccidials and of resistance to anticoccidial drugs; chemical additives to feed to reduce salmonella contamination; the biochemistry of hemagglutinating, adenoviruses; and research on lymphoproliferative disease of turkeys.

2. Disease Problems

At the end of the review, all participants were asked to score program areas using the same criteria. The scores are summarized in Table 5.

The grand average score indicates a need for additional research effort in many areas but most specifically in colibacillosis (particularly in turkeys), influenza, mycoplasmosis, ornithosis, infectious bronchitis and immunogenetics. The score confirmed that there was no need to initiate research in fowl pox.

TABLE 4. Summary of Scores of Research Approaches

ject ea	Proj No.	Contributing Scientists																Consultants												Others							Grand Average
		Augustine	Bacon	Beard	Brugh	Cheville	Crittenden	Deaton	Fadly	Pier	Rhoades	Rimler	Ruff	Sharma	Tessler	Witter	Yoder	Ave. Scientist	Kleven	Edgar	Calnek	Pomeroy	Ave. Univ.	Craig	Hughes	Ave. Industry	Hook	Price	Peterson	Ave. APHIS	Ave. Consultant	King	O'Berry	Lebsock	Herlich	Patterson	Purchase	Ave. Other	
hosis	1	2	3	2	2	3	2	3	3	2	2	3	2	3		2	2	2.4	3	2		2	2.3	2	2	2.0	0	2	3	1.7	2.0	1	1	3	2	2	3	2.0	2.1
	2	3	2	3	3	4	3	3	3	3	3	3	4	3		3	3	3.4	3	3		3	3.0	3	4	3.5	1	2	4	2.3	2.9	2	3	3	3	2	4	3.4	3.2
	3	2	3	4	2	3	3	2	2	3	3	3	3	2		4	4	2.9	2	2		4	2.7	2	3	2.5	2	2	4	2.7	2.6	1	2	3	4	4	3	2.8	2.8
	x	2.3	2.7	3.0	2.3	3.3	2.7	2.7	2.7	2.7	2.7	3.0	3.0	2.7		3.0	3.0	2.9	2.7	2.3		3.0	2.8	2.3	2.7	2.5	1.0	2.0	3.7	2.2	2.4	1.3	2.0	3.0	3.0	2.7	3.3	2.7	2.7
acillosis	1	1	2	3	2	3	3	2	3	1	2	3	2	2	1	3	2	2.2	1	1		2	1.3	2	3	2.5	2	2	3	2.3	2.0	1	1	3	3	2	2	2.0	2.1
	2	1	2	2	2	2	2	2	3	2	2	3	1	2	2	1	3	2.0	1	2		2	1.7	2	1	1.5	1	1	3	1.7	1.6	2	2	2	2	1	1	1.7	1.8
	3	3	3	3	4	4	3	2	3	3	4	3	2	3	2	3	3	3.0	2	3		2	2.3	2	3	2.5	3	2	3	2.7	2.5	3	3	3	3	3	3	3.0	2.8
	4	3	2	2	2	2	3	2	2	3	2	2	2	2	2	2	4	2.3	2	1		1	1.3	2	1	1.5	2	2	3	2.3	1.7	2	2	2	2	1	2	1.8	1.9
	x	2.0	2.3	2.5	2.5	2.8	2.8	2.0	2.8	2.2	2.5	2.8	1.8	2.3	1.8	2.3	3.0	2.5	1.5	2.2		2.2	2.1	2.0	2.0	2.0	2.0	1.8	3.0	2.3	2.4	2.0	2.0	2.5	2.5	1.8	2.0	2.1	2.2
urellosis	1	3	3	1	2	3	2	3	3	2	1	1	1	2	2	2	2	2.1	1	1		2	1.3	2	4	3.0	2	2	2	2.0	2.1	2	2	2	3	1	1	1.7	2.0
	2	2	4	2	4	2	3	3	2		3	1	2	2	2	4	3	2.6	2	2		2	2.0	3		3.0	4	4	2	3.3	2.8	2	3	1	3	3	4	2.2	2.5
	3	1	2	4	3	3	3	3	4	3	3	2	2	3	1	3	3	2.7	3	3		2	2.7	3	4	3.5	3	3	3	3.0	3.1	2	1	2	2	4	3	2.3	2.7
	4	3	3	4	5	5	3	3	2	3	3	3	3	2	2	4	4	3.3	3	2		3	2.7	3	4	3.5	3	2	3	2.7	3.0	4	3	3	3	2	3	3.0	3.1
	5	3	2	3	2	3	2	3	2	2	2	1	1	3	1	2	2	2.1	2	3		2	2.3	2	4	3.0	1	2	3	2.0	2.4	2	3	3	2	4	2	2.7	2.4
	x	2.4	2.8	2.8	3.2	3.2	2.6	3.0	2.6	1.8	2.6	1.6	1.8	2.4	1.6	3.0	2.8	2.6	2.2	2.2		2.2	2.2	2.6	4.0	3.2	2.6	2.6	2.2	2.6	2.7	2.9	2.4	2.2	2.6	2.8	2.6	2.4	2.6
/ Airsac- itis	1	3	3	2	3	3	3	2	3	2	3	2	2	3	1	3	2	2.5	2	3	4	2	2.8	4	3	3.5	4	4	2	3.3	3.2	2	1	2	3	3	4	2.5	2.7
	2	1	4	3	1	3	2	2	2	2	2	3	2	1	4	2	2	2.3	2	1	3	1	1.8	2	1	1.5	2	2	2	2.0	2.5	2	2	2	1	1	3	1.8	2.2
	3	3	2	4	2	3	3	2	3	3	2	3	3	3	2	2	3	2.7	2	3	3	2	2.5	3	2	2.5	3	3	3	3.0	2.7	3	3	3	3	3	3	3.0	2.8
	4	3	3	4	2	3	2	1		3	3	3	3	3	2	4	2	2.7	3	2	4	1	2.5	2	3	2.5	3	2	3	2.7	2.6	2	3	3	2	2	3	2.5	2.6
	5	3	2	2	1	4	2	1	2	3	2	3	1	2	1	2	3	2.1		3	2	1	2.0	2	4	3.0	3	2	3	2.7	2.6	1	3	3	2	2	2	2.2	2.3
	x	3.2	2.8	3.0	1.8	3.2	2.4	1.6	2.5	2.6	2.4	2.8	2.2	2.4	3.0	2.6	2.4	2.5	2.3	2.4	3.2	1.4	2.3	2.6	2.6	2.6	3.0	2.6	2.6	2.7	2.7	2.0	2.4	2.6	2.2	2.2	3.0	2.4	2.5
oxicoses	1	3	3	2	3	3	3	2	2	1	2	3	2	2	1	3	2	2.3	3	3		2	2.7	3	3	3.0	3	3	4	3.3	3.0	1	3	2	2	2	3	2.2	2.5
	2	3	2	4	5	5	3	2	3	3	3	2	2	3	2	3	2	2.9	4	1		3	2.7	2	4	3.0	2	3	4	3.0	2.9	3	3	3	4	2	4	3.2	3.0
	3	3	3	3	2	3	3	1	2	3	2	2	2	3	2	2	3	2.4	3	2		2	2.3	2	3	2.5	3	2	3	2.7	2.5	2	2	3	2	3	2	2.0	2.3
	4	2	3	4	3	2	3	1	2	2	2	3	3	3	4	2	3	2.6	2	1		3	2.0	3	2	2.5	2	1	4	2.7	2.4	2	1	3	2	3	3	2.5	2.5
	x	2.8	2.8	3.3	3.3	3.3	3.0	1.5	2.3	2.3	2.3	2.5	2.3	2.8	2.3	2.5	2.5	2.6	3.0	1.8		2.5	2.4	2.5	3.0	2.8	2.5	2.3	3.8	2.9	2.7	2.0	2.3	2.8	2.5	2.3	3.0	2.5	2.6
es	1	1	2	3	2	3	2	1	4	2	2	1	1	1	2	4	2	2.1	2	2	4	1	2.3	3	4	3.5	2	2	1	1.7	3.3	2	4	2	3	2	2	2.5	2.6
	2	1	2	4	3	4	2	1	2	2	2	3	2	1	4	2	4	2.4	1	2		1	1.3	3	2	2.5	2	2		2.0	1.9	1	3	2	5	2	3	2.7	2.3
	x	1.0	2.0	3.5	2.5	3.5	2.0	1.0	3.0	2.0	2.0	2.0	1.5	1.0	3.0	3.0	3.0	2.3	1.5	2.0	4.0	1.0	1.8	3.0	3.0	3.0	2.0	2.0	1.0	1.9	2.2	1.5	3.5	2.0	4.0	2.0	2.5	2.6	2.4

TABLE 4 (continued)

Lab	Project Area	Proj No.	Contributing Scientists																Consultants												Others							Grand Average
			Augustine	Bacon	Beard	Brugh	Cheville	Crittenden	Deaton	Fadly	Pier	Rhoades	Rimler	Ruff	Sharma	Tessler	Witter	Yoder	Ave. Scientist	Kleven	Edgar	Calnek	Pomeroy	Ave. Univ.	Craig	Hughes	Ave. Industry	Hook	Price	Peterson	Ave. APHIS	Ave. Consultant	King	O'Berry	Lebsock	Herlich	Patterson	Purchase	Ave. Other	
SEPRL	Avian Influenza	1	4	4	3	3	4	3	3	3	3	3	3	3	3	5	5	3	3.4	2	3	3	3	2.8	3	3	3.0	5	2	5	4.0	3.3	3	3	3	4	3	4	3.3	3.3
		2	3	3	3	3	3	3	3	4	2	3	4	2	3	2	2	2	2.8	3	1	2	3	2.3	2	2	2.0	1	2	3	2.0	2.1	2	3	3	3	2	1	2.3	2.4
		3	1	1	1	1	1	3	1	4	1	2	3	2	1	2	2	1	1.7	1	2	1	1	1.3	1	1	1.0	1	1	1	1.0	1.1	1	1	2	2	1	1	1.3	1.4
		4	2	2	2	2	2	1	1	2	3	2	1	2	3	2	1	2	1.9	1	2	1	1	1.3	2	2	2.0	1	1	2	1.3	1.5	1	2	3	2	1	2	1.8	1.7
		x	2.5	2.5	2.3	2.3	2.5	2.5	2.0	3.3	2.3	2.5	2.8	2.3	2.5	2.8	2.3	2.0	2.5	1.8	2.0	1.8	2.0	1.9	2.0	2.0	2.0	2.0	1.5	2.8	2.1	2.8	1.8	2.3	3.0	2.8	1.8	2.0	2.2	2.2
	Salmonellosis	1	3	4	4	2	4	3	2	3	3	3	3	4	5	4	3	3	3.3	3	3		2	2.7	2	4	3.0	4	2	1	2.3	2.7	2	2	3	5	4	4	3.3	3.1
		2	1	3	2	1	3	2	2	5	2	3	3	2	2	1	1	2	2.2	1	2		1	1.3	3	4	3.5	1	2	1	1.3	2.0	2	1	2	4	2	1	2.0	2.1
		3	2	2	3	2	3	2	2	4	3	3	4	3	3	1	2	3	2.6	3	3		2	2.7	2	3	2.5	3	3	3	3.0	2.7	3	3	4	3	2	3	3.0	2.8
		4	4	3	2	4	2	2	2	3	3	2	3	3	4	2	2	4	2.8	2	2		3	2.3	1	4	2.5	3	3	4	3.3	2.7	3	4	2	3	2	2	2.7	2.7
		5	4	4	3	2	5	3	2	1	2	4	5	4	5	2	2	5	3.3	5	4		3	4.0	4	2	3.0	4	5	3	4.0	3.7	3	3	5	3	1	3	3.0	3.3
		6	3	2	2	2	3	2	2	2	4	3	2	2	2	2	1	3	2.3	2	5		2	3.0	2	4	3.0	1	2		1.5	2.5	1	3	2	4	1	1	2.0	2.3
		7	2	3	2	2	2	3	2	5	2	3	4	3	2	4	1	3	2.7	2	4			3.0	2	4	3.0	2	2	2	2.0	2.7	1	2	2	4	2	2	2.2	2.5
		8	1	3	3	3	1	2	2	2	3	2	3	1	2	2	2	4	2.3	1	5		2	2.7	1	3	2.0	1	2	2	1.7	2.1	1	1	2	4	2	1	1.8	2.7
		x	2.5	3.0	2.6	2.3	2.9	2.4	2.0	3.1	2.8	2.9	3.4	2.8	3.1	2.3	1.8	3.4	2.7	2.4	3.5		2.1	2.7	2.1	3.5	2.8	2.4	2.6	2.3	2.4	2.6	2.0	2.4	2.8	3.8	2.0	2.1	2.5	2.6
	Inf. Bronchitis	1	2	3	2	3	3	2	2	2	2	3	3	1	3	2	2	2	2.3	1	2	2	3	2.0	1	2	1.5	2	3	3	2.7	2.1	1	2	3	1	1	1	1.5	2.0
		2	2	2	1	1	1	2	3	2	3	2	3	2	2	1	3	1	1.9	1	1	3	1	1.5	2	1	1.5	1	2	1	1.3	1.4	1	3	2	2	3	3	2.3	1.9
		3	3	2	2	2	3	4	3	3	3	3	3	1	3	2	2	2	2.6	1	1	2	2	1.5	3	3	3.0	2	2	3	2.3	2.1	3	3	3	1	2	1	2.2	2.3
		4	2	3	3	3	3	3	3	3	3	3	3	3	5	3	3	3	3.1	1	3	3	3	2.5	3	3	3.0	2	2	2	2.0	2.4	3	3	3	3	3	3	3.0	2.8
		5	1	3	2	1	2	3	3	3	1	2	3	2	3	2	3	2	2.3	1	1	4	3	2.3	2	2	2.0	1	1	1	1.0	2.0	2	3	3	3	3	4	3.0	2.4
		x	2.0	2.6	2.0	2.0	2.4	2.8	2.8	2.6	2.4	2.6	3.0	1.8	2.8	2.4	2.6	2.0	2.4	1.0	1.6	2.8	2.4	2.0	2.2	2.2	2.2	1.6	2.0	2.0	1.9	2.0	2.0	2.8	2.8	2.0	2.4	2.4	2.4	2.3
	Mycoplasma	1	2	3	3	1	2	2	2	2	2	1	2	1	2	2	1	2	1.9	1	1	2	3	1.8	3	1	2.0	1	1	2	1.3	1.7	2	2	2	1	3	2	2.0	1.9
		2	2	3	2	3	4	3	1	3	3	2	3	2	3	1	3	3	2.6	2	3	4	1	2.5	3	2	2.5	3	2	2	2.3	2.4	2	3	3	3	2	1	2.3	2.5
		3	3	2	3	2	3	3	1	2	2	2	2	1	3	2	3	4	2.4	2	3	3	2	2.5	3	2	2.5	2	2	3	2.3	2.4	2	1	2	1	2	2	1.7	2.3
		4	3	4	2	2	3	3	2	3	3	3	2	2	2	1	2	3	2.5	4	2	3	2	2.8	2	3	2.5	3	4	3	3.3	2.9	3	3	3	1	1	3	2.3	2.6
		5	3	3	4	4	3	2	2	4	3	3	4	2	4	4	3	5	3.3	2	2	2	1	1.8	2	3	2.5	2	2	2	2.0	2.0	3	4	3	3	4	3	3.3	2.9
		6	3	3	1	1	3	2	2	2	2	2	2	1	2	2	2	2	2.0	3	1	2	2	2.0	1	1	1.0	4	5	2	3.7	2.3	1	3	3	2	1	2	2.0	2.1
		7	4	2	2	2	3	2	2	2	3	3	3	2	2	2	1	4	2.4	3	2	2	2	2.2	1	2	1.5	4	5	2	3.7	2.6	1	3	3	2	2	2	2.2	2.4
		x	2.9	2.8	2.4	2.1	3.0	2.4	1.7	2.6	2.6	2.3	2.6	1.6	2.6	2.0	2.1	3.3	2.4	2.4	2.0	2.6	1.8	2.2	2.1	2.0	2.1	2.7	3.0	2.3	2.7	2.3	2.0	2.7	2.7	1.9	2.1	2.1	2.3	2.4
	Newcastle Disease	1	3	2	2	2	3	2	1	3	3	3	3	2	3	2	3	3	2.5	1	3	2	3	2.3	2	3	2.5	1	1	2	1.3	2.0	2	3	3	3	3	3	2.8	2.4
		2	3	4	3	3	2	2	2	4	3	3	2	3	3	4	4	2	2.9	2	2	3	2	2.3	2	2	2.0	2	2	2	2.0	2.1	2	3	3	4	3	4	3.2	2.7
		3	2	4	1	1	1	4	2	3	2	2	2	2	2	4	3	2	2.3	2	1	2	2	1.8	2	2	2.0	1	2	1	1.3	2.0	1	2	2	1	2	3	1.8	2.0
		4	2	3	2	2	4	3	2	3	2	2	4	2	2	2	3	2	2.5	1	2	3	1	1.8	2	3	2.5	1	1	2	1.3	1.8	2	1	2	2	2	3	2.0	2.1
		5	1	1	2	3	2	1	1	2	1	1	1	1	1	2	2	2	1.5	1	2	1	1	1.3	1	2	1.5	0	1	1	0.7	1.1	1	2	2	2	1	3	1.8	1.5
		x	2.2	2.8	2.0	2.2	2.4	2.4	1.6	3.0	2.2	2.2	2.4	2.0	2.2	2.8	3.0	2.2	2.3	1.4	2.0	2.2	1.8	1.9	1.8	2.4	2.1	1.0	1.4	1.6	1.3	1.8	1.6	2.2	2.4	2.4	2.2	3.2	2.3	2.1

TABLE 4 (continued)

Lab	Project Area	Proj No.	Contributing Scientists																	Consultants												Others							Grand Average
			Augustine	Bacon	Beard	Brugh	Cheville	Crittenden	Deaton	Fadly	Pier	Rhoades	Rimler	Ruff	Sharma	Tessler	Witter	Yoder	Ave. Scientist	Kleven	Edgar	Calnek	Pomeroy	Ave. Univ.	Craig	Hughes	Ave. Industry	Hook	Price	Peterson	Ave. APHIS	Ave. Consultant	King	D'Berry	Lebsock	Herlich	Patterson	Purchase	Ave. Other	
API	Coccidiosis	1	1	4	3	2	1	3	2	2	3	3	2	1	3	2	3	2	2.3	2	1		2	1.7	1	3	2.0	2	2	4	2.7	2.1	2	3	1	2	2	1	1.8	2.1
		2	3	3	3	4	3	2	4	3	4	3	3	3	2	4	4	4	3.3	3	2		3	2.7	3	4	3.5	4	4	4	4.0	3.4	2	4	3	2	3	3	2.8	3.3
		3	1	2	4	3	4	4	3	3	3	3	3	2	3	4	4	3	3.1	2	1		3	2.0	2	4	3.0	3	3	4	3.3	2.8	2	4	2	2	3	2	2.5	2.7
		4	3	4	3	3	5	4	3	2	3	3	3	2	3	4	4	3	3.3	3	2		3	2.7	3	4	3.5	3	3	4	3.3	3.1	2	3	2	2	5	3	2.8	3.0
		5	1	2	3	3	5	2	3	2	2	2	1	1	3	2	4	3	2.4	3	2		3	2.7	3	3	3.0	2	2	4	2.7	2.8	2	2	2	3	3	4	2.7	2.6
		6	3	4	4	2	3	2	3	2	3	4	3	1	3	2	3	3	2.8	4	1		2	2.3	2	3	2.5	2	2	4	2.7	2.5	2	3	3	3	1	2	2.3	2.5
		7	3	3	3	2	2	2	3	1	3	3	3	1	1	4	2	2	2.4	3	1		4	2.7	3	3	3.0	1	1	4	2.0	2.5	1	1	3	3	2	2	2.0	2.3
		8		3	2	2	4	2	2	2	4	3	3	2	2	2	4	3	2.7	4	2		3	3.0	3	4	3.5	2	2	4	2.7	3.0	2	4	4	2	2	2	2.7	2.8
		9	3	3	4	2	4	2	2	2	3	3	3	2	3	4	3	2	2.8	4	1		1	2.0	2	2	2.0	3	3	4	3.3	2.5	2	3	3	3	2	4	2.8	2.7
		10	1	2	4	3	4	3	3	3	3	3	2	3	3	2	4	3	2.9	2	2		2	2.0	3	4	3.5	4	4	3	3.7	3.0	2	3	3	3	4	3	3.0	3.0
		11	3	3	4	4	2	3	3	1	3	4	2	3	4	2	4	3	3.0	2	3		3	2.7	3	2	2.5	3	3	2	2.7	2.6	1	4	2	3	4	3	2.8	2.8
		12	3	4	5	3	3	3	4	4	4	3	3	3	3	4	4	4	3.6	3	3		3	3.0	4	3	3.5	4	4	3	3.7	3.4	3	4	4	4	4	3	3.7	3.6
		13	1	2	3	2	3	2	3	3	2	3	3	2	1	4	2	4	2.5	3	3		3	3.0	3	2	2.5	3	3	3	3.0	2.9	2	3	3	3	3	2	2.7	2.7
		14	1	3	3	1	3	3	3	2	3	3	2	2	2	2	2	2	2.3	2	2		2	2.0	3	2	2.5	1	1	1	1.0	1.8	2	2	4	3	2	2	2.5	2.2
		15	1	3	4	2	2	2	3	3	2	3	1	1	4	4	3	3	2.6	2	3		3	2.7	4	2	3.0	2	2	2	2.0	2.5	2	3	1	3	3	2	2.3	2.5
		16	1	4	3	3	3	2	3	2	2	3	1	2	4	2	2	3	2.5	2	2		3	2.3	2	3	2.5	2	2	1	1.7	2.1	2	3	1	3	3	3	2.5	2.3
		17	3	2	3	2	4	1	3	1	3	2	3	3	1	4	1	4	2.5	2	3		1	2.0	2	3	2.5	4	2	1	2.3	2.3	2	3	2	2	4	3	2.7	2.5
		18	1	2	4	3	3	2	3	2	3	3	3	2	3	4	2	4	2.8	2	2		3	2.3	3	3	3.0	4	1	3	2.7	2.6	2	3	2	3	4	4	3.0	2.8
		19	1	2	2	1	2	2	1	1	3	3	3	1	1	4	1	2	1.9	1	2		2	1.7	2	2	2.0	1	1	3	1.7	1.8	1	3	1	2	1	1	1.5	1.7
		20	1	1	2	2	3	2	1	1	3	3	3	1	1	4	1	3	2.0	1	2		2	1.7	2	2	2.0	1	2	3	2.0	1.9	1	3	1	2	1	2	1.7	1.8
		21	5	3	3	4	3	2	2	4	4	3	3	1	3	2	3	3	3.0	2	2		4	2.7	3	4	3.5	5	3	3	3.7	3.3	3	4	4	4	2	3	3.3	3.2
		22	5	4	2	2	4	2	4	5	4	4	5	4	3	4	5	4	3.8	4	3		4	3.7	4	4	4.0	1	2		1.5	2.1	3	2	3	4	3	5	3.3	3.4
		23	5	1	1	2	3	2	1	5	4	2	2	1	1	1	5	5	2.6	2	3		2	2.7	2	2	2.0	2	2	1	1.7	2.0	2	3	4	5	3	1	3.0	2.4
		24	3	3	2	2	5	3	4	5	2	3	3	1	5	4	3	4	3.3	3	1		3	2.3	2	2	2.0	5	3	3	3.7	2.8	1	3	4	2	4	5	3.2	3.1
		x	2.3	2.8	3.1	2.5	3.3	2.4	2.8	2.5	3.0	3.0	2.6	1.9	2.6	3.1	3.0	3.2	2.8	2.5	2.0		2.7	2.4	2.7	2.9	2.8	2.72	4	3.0	2.7	2.6	1.9	3.0	2.6	2.8	2.8	2.7	2.6	2.7
RPRL	Lymphoid Leukosis	1	3	3	2	3	1	3	2	3	2	3	2	3	2	2	3	3	2.5	3	3	2	2	2.5	2	2	2.0	1	2	3	2.0	2.2	2	1	3	3	4	3	2.7	2.5
		2	4	1	3	3	3	3	3	3	3	3	4	3	3	2	1	3	2.8	2	3	3	3	2.8	1	4	2.5	3	1	4	2.7	2.7	3	3	3	4	3	3	3.1	2.9
		3	2	2	2	1	2	2	1	2	2	2	2	2	2	4	1	2	1.9	1	2	1	2	1.5	2	2	2.0	3	2	2	2.3	2.2	2	2	3	3	2	1	2.2	2.1
		4	2	2	4	2		3	3	3	3	3	3	2	3	2	3	3	2.7	1	2	3	3	2.3	2	3	2.5	2	2	2	2.0	2.2	2	3	2	2	3	2	2.0	2.3
		5	1	2	4	3	4	3	3	3	4	2	4	1	1	4	3	2	2.6	3	2	4	3	3.0	3	2	2.5	4	3	2	3.0	2.9	3	3	2	1	2	4	2.5	2.7
		6	2	2	4	2		2	3	2	2	3	3	2	3	2	2	3	2.5	2	2	3	2	2.3	3	3	3.0	3	3	3	3.0	2.7	3	3	1	2	3	2	1.8	2.3
		7	2	2	2	1	4	1	3	1	4	2	2	1	3	4	1	2	2.2	2	2	3	2	2.3	2	3	2.5	4	2	3	3.0	2.6	3	2	1	2	1	1	1.7	2.2
		8	3	3	3	3	3	3	2	2	2	3	2	3	4	2	4	3	2.8	2	3	4	3	3.0	2	2	2.0	2	2	4	2.7	2.7	2	3	3	4	3	3	3.0	2.8
			2.4	2.1	3.0	2.3	2.8	2.5	2.5	2.4	2.8	2.6	2.8	2.1	2.6	2.8	2.3	2.6	2.5	2.0	2.4	2.9	2.5	2.5	2.1	2.6	2.4	2.82	.2	2.9	2.6	2.5	2.5	2.5	2.3	2.6	2.6	2.4	2.5	2.5
	nf Bursal Disease	1		3	2	2	2	2	2	1	2	2	1	2	1	2	3	2	2.0	1	2	2	2	1.8	2	2	2.0	1	1	2	1.3	1.7	2	1	3	3	3	2	2.7	2.1
		2		2		3	3	4	3	1	4	2	4	2	5	3	3	3	3	4	1	1	2.3	3	1	2.0	2	2	3	2.3	2.6	3	3	3	4	4	3	3	2.9
		3	3	3		2	3	2	3	2	3	3	3	2	2	2	3	3	2.8	1	3	2	3	2.3	2	2	2.0	1	2	4	2.3	1.8	2	2	1	3	3	1	2	2.3
		x	3	3	3.3	2.3	2.7	2.7	2.7	1.3	3.0	3.0	2.0	3	1.7	3.0	3.0	1.7	2.6	1.3	3.0	1.7	2.0	2.1	2.3	1.7	2.0	1.3	1.73	.0	2.0	2.0	2.3	2.0	2.0	3.3	3.3	2.0	2.7	2.3
	Marek's disease	1		3		1	3	2	1	3	2	3	3	2	2	2	3	2	2.3	1	3	1	2	1.8	1	3	2.0	2	3	3	2.7	2.1	2	2	3	1	2	3	2	2.3
		2	3	2	2	2	3	2	2	3	2	2	2	2	2	2	1	2	2.1	1	2	2	1	1.5	1	2	1.5	3	1	1	1.7	1.6	2	3	3	3	2	2	2.5	2.1
		3	4	2	3	4	4	3	2	3	3	3	3	2	4	2	3	3	3.1	2	2	3	2	2.5	1	3	2.0	1	1	4	2.0	2.2	5	3	3	3	3	3	3.3	3.0
		4	2	3	3	2	3	3	3	3	4	1	3	3	2	2	3	2	2.5	2	3	3	3	2.8	2	2	2.0	1	3	3	2.3	2.4	2	4	2	3	3		2	2.6
		5	2	3	4	3	2	4	3	3	2	2	2	2	2	3	3	2	2.8	2	4	3	3	3.0	2	2	2.0	3	2	2	2.7	2.7	3	3	2	3	3			2.7
		6	1	1	3	1	2	1	2	1	4	3	2	1	3	2	1	2	1.7	2	3	1	3	2.3	1	2	1.5	2	2	2	2.	2.	2	2	2	3	2	4	2.7	2.2
			3	2	1	3	3	2	2	4	3	3	2	2	3	3	3	3	2.2	3	3	1	3	2.6	2	2	2.0	1	3	3	2.	2.	3	3	1	4	1	3	2.7	2.4
			2.4	2.4	2.6	2.3	2.6	2.4	2.1	2.7	3.7	3.7	2.3	2.1	2.4	2.1	2.1	2.6	2.4	2.0	2.7	1.8	2.4	2.4	2.3	1.9	1.6	2.7	2	2.	2.2	2.	2.9	2.	2.7	2.4	2.7	2.8	2.4	2.4

TABLE 4 (continued)

Lab	Project Area	Proj No.	Contributing Scientists																Consultants												Others							Grand Average
			Augustine	Bacon	Beard	Brugh	Cheville	Crittenden	Deaton	Fadly	Pier	Rhoades	Rimler	Ruff	Sharma	Tessler	Witter	Yoder	Ave. Scientist	Kleven	Edgar	Calnek	Pomeroy	Ave. Univ.	Craig	Hughes	Ave. Industry	Hook	Price	Peterson	Ave. APHIS	Ave. Consultant	King	O'Berry	Lebsock	Herlich	Patterson	Purchase	Ave. Other	
RPRL	Adenoviruses	1	3	2	3	1	3	2	3	1	2	2	3	3	1	1	2	2	2.1	1	2	1	1	1.3	3	2	2.5	1	3	1	1.7	1.7	2	1	2	1	2	1	1.5	1.8
		2	3	3	4	4	4	3	3	2	3	3	4	3	2	2	3	3	3.1	3	3	3	4	3.3	3	3	3.0	3	3	5	3.7	3.0	3	3	3	4	3	3	3.2	3.1
		3	3	3	2	2	3	2	3	3	4	3	5	4	2	4	3	2	3.0	3	3	2	4	3.0	1	4	2.5	3	1	3	2.3	2.7	2	3	2	2	2	2	2.2	2.6
		4	2	3	3	3	3	3	3	2	3	2	2	2	1	2	2	3	2.4	2	2	2	2	2.0	2	2	2.0	4	2	5	3.7	2.6	2	2	3	4	3	2	2.7	2.6
		5	4	3	4	3	3	3	3	3	3	3	4	2	3	2	3	4	3.1	2	2	2	3	2.3	4	3	3.5	2	3	5	3.3	2.9	2	3	4	1	3	2	2.5	2.8
		6	3	2	2	3	2	3	3	2	4	3	5	3	2	2	2	4	2.5	3	3	2	3	2.8	2	4	3.0	2	1	3	2.0	2.5	2	3	4	1	2	1	2.2	2.4
		7	2	3	2	1	3	2		2	2	3	5	4	4	1	2	3	2.6	1	2	1	2	1.5	2	4	3.0	2	1	1	1.3	1.8	3	3	4	4	2	2	3.0	2.5
		x	2.9	2.7	2.9	2.4	3.0	2.6	3.0	2.1	3.0	2.7	4.0	3.0	2.1	2.0	2.4	3.0	2.7	2.1	2.4	1.9	2.7	2.3	2.4	3.1	2.8	2.4	2.0	3.3	2.6	2.5	2.3	2.6	3.1	2.4	2.4	1.9	2.5	2.6
	Immunogenetics	1	2	2	4	2	3	2	3	3	2	3	1	3	3	2	3	2	2.5	2	1	2	3	2.0	2	3	2.5	2	2	3	2.3	2.2	2		2	2	4	4	2.8	2.5
		2	1	3	4	2	3	3	3	3	1	3	1	2	4	2	4	3	2.6	2	2	3	2	2.3	3	3	3.0	2	3	3	2.7	2.6	3		2	3	3	4	3.0	2.7
		3	2	2	3	2	3	2	3	2	3	3	1	3	3	4	2	3	2.1	3	2	2	3	2.5	2	4	3.0	3	2	3	2.7	2.7	4		3	3	3	2	3.0	2.6
		x	1.7	2.3	3.7	2.0	3.0	2.3	3.0	2.7	2.0	3.0	1.0	2.7	3.3	2.7	3.0	2.7	2.6	2.3	1.7	2.3	2.7	2.3	2.3	3.3	2.8	2.3	2.3	3.0	2.5	2.5	3.0		2.3	2.7	3.3	3.3	2.9	2.6
	Other Neoplasms	1	3	2	3	2	3	3	3	2	3	2	3	3	3	2	2	3	2.6	2	2	3	3	2.5	2	3	2.5	1	2	3	2.0	2.3	2		3	1	3	3	2.4	2.4
		2	3	3	4	3	3	2	3	3	2	3	3	3	2	4	3	3	2.9	3	3	3	3	3.0	3	2	2.5	3	3	3	3.0	2.9	2		2	3	2	2	2.2	2.7
		3	2	3	4	2	3	3	3	3	2	3	3	2	3	2	3	3	2.8	3	2	2	4	3.0	2	3	2.5	3	2	3	2.7	2.7	3		2	2	3	3	2.6	2.7
		4	4	3	2	1	4	4	2	1	3	5	4	4	2	4	2	3	2.7	3	3	2	2	2.5	1	4	2.5	5	1	3	3.0	2.6	2		3	5	4	3	3.4	2.9
		5	2	2	2	1	3	3	3	3	2	3	3	3	2	4	2	2	2.5	2	2	3	1	2.0	1	2	1.5	2	2	2	2.0	2.7	2		3	3	2	2	2.4	2.5
		6	2	3	3	2	3	2	2	3	2	3	3	3	3	2	2	3	2.6	3	4	4	2	3.3	2	4	3.0	3	3	2	2.7	3.0	2		3	5	4	4	3.6	3.1
		x	2.7	2.7	3.0	1.8	3.2	2.8	2.7	2.5	2.5	3.0	3.2	3.0	2.5	3.0	2.3	2.8	2.7	2.7	2.7	2.8	2.5	2.7	1.8	3.0	2.4	2.8	2.2	2.7	2.6	2.6	2.2		2.7	3.2	3.0	2.8	2.8	2.7
Grand Average			2.1	2.7	2.8	2.3	3.0	2.5	2.4	2.6	2.5	2.7	2.7	2.2	2.5	2.6	2.6	2.8	2.6	2.2	2.3	2.5	2.2	2.3	2.3	2.7	2.5	2.3	2.2	2.7	2.3	2.4	2.1	2.6	2.6	2.7	2.5	2.5	2.5	2.5

Project Area	Contributing Scientists																	Consultants														Others						Grand Average
	Augustine	Bacon	Beard	Brugh	Cheville	Crittenden	Deaton	Fadly	Pier	Rhoades	Rimler	Ruff	Sharma	Tessler	Witter	Yoder	Ave. Scientist	Kleven	Edgar	Calnek	Pomeroy	Ave. Univ.	Craig	Hughes	Ave. Industry	Hook	Price	Peterson	Ave. APHIS	Ave. Consultant	King	O'Berry	Lebsock	Herlich	Patterson	Purchase	Ave. Other	
Chickens																																					
Coccidiosis	2	3	3	3		3	3	3	3	3	3	2	3	2	4	3	2.9	4		3	3	3.3	3	4	3.5	2	4	3	3.0	3.3	3			3	3	3	3.0	3.0
Salmonellosis	4	4	1	3		2	2	3	4	3	3	5	3	3	3	2	3.0	3	3	3	2	2.8	2	3	2.5	3	1	2	2.0	2.4	2			4	2	2	2.5	2.8
Pasteurellosis	3	3	3	3		3	2	3	2	3	3			2	5	3	2.9	3	2	3	4	3.0	2	3	2.5	3	2	3	2.7	2.8	3				3	3	3.0	2.9
Colibacillosis	1	2	2	2		2	1	4	3	3			3		3	3	2.4	1	2	3	3	2.3	1	2	1.5	3	2	3	2.7	2.2	3				3	2	2.7	2.4
Mycoses	2	1	4	3		3	1	4	2	3	2	3	2		5	3	2.7	3	2	4	3	3.0	3	4	3.5	3	1	4	2.7	3.0	2			3	3	3	2.8	2.9
Mycotoxicoses	3	1	2	4		3		3	1	3	2	2	2	3	2	3	2.4	3	2	4	3	3.0	2	4	3.0	3	2	3	2.7	2.9	2			3	2	3	2.5	2.6
Mycoplasmosis	3	3	2	3		3	2	3		3	2		3	2	3	3	2.7	2	2	3	2	2.5	1	4	2.5	3	2	2	2.3	2.3	2			3	2	3	2.5	2.5
Fowl Pox	3	3	5	3		3	3	3	5	3	3	5	3	3		3	3.4	3	3		5	3.7	3	4	3.5	4	3	4	3.7	3.6	3				3	3	3.0	3.6
Adenoviruses	4	3	4	3		3	3	2	3	4	4	3	3	3	2	2	3.1	3	2	5	4	3.5	3	4	3.5	1	1	4	2.0	3.0	3			3	3	3	3.0	3.0
Inf. Bursal Disease	2	3	3	4		3	2	2	2	3	3	3	2	3	3	2	2.7	2	3	4	3	3.0	2	2	2.0	2	2	2	2.0	2.4	2			3	3	3	2.8	2.6
Inf. Bronchitis	3	4	1	2		3	2	3	3	3	3	2	3	3	3	2	2.7	1	1	3	3	2.0	2	3	2.5	2	2	2	2.0	2.1	2			2	3	3	2.5	2.5
Influenza	4	2	2	3		3	3	3	3	3	3	3	3	3	3	2	2.9	3	2	3	4	3.0	2	2	2.0	1		3	2.0	2.5	2			3	1	3	2.3	2.7
Newcastle Disease	3	4	3	3		3	2	2	2	3	3	2	3	2	3	3	2.7	2	2	2	3	2.3	2	4	3.0	1	2	2	1.7	2.2	2			3	3	4	3.0	2.6
Lymphoid Leukosis	3	3	4	3		1	2	2	3	3	3	4	3	2	3	3	2.8	4	3	2	4	3.3	3	4	3.5	3	3	3	3.0	3.2	3			3	1	4	2.8	2.9
Marek's Disease	3	3	3	4		3	2	2	3	3	3	2	3	2	3	3	2.8	2	3	3	3	2.8	2	3	2.5	3	3	2	2.7	2.7	4			3	3	3	3.3	2.8
Oth. Neoplasms (SCC,RE)	4	3	4	3		3	2	3	3	3	3		3	3	3	2	3.0	3	3	3	2	2.8	3	3	3.0	4	2	3	3.0	2.9	3			3	3	3	3.0	3.0
Immunogenetics	3	3	3	3		2	2	3	1	4	2	3	3	2	2	3	2.6	3	2	2	3	2.5	3	3	3.0	2	1	3	2.0	2.4	3			3	3	3	3.0	2.6
$\bar{x}$	2.9	2.8	2.9	3.1		2.7	2.1	2.8	2.7	3.1	2.8	3.0	2.8	2.5	3.1	2.6	2.8	2.6	2.3	3.1	3.2	2.9	2.3	3.3	2.8	2.5	2.1	.8	2.5	2.7	2.6			3.0	2.6	3.0	2.8	2.8
Turkeys																																					
Coccidiosis	2	4	4	2		3	3	2	3	3	3	2	3	2	4	2	2.8	3		3	3	3.0	2	3	2.5	2	1	3	2.0	2.5	2			3	3	3	2.8	2.7
Salmonellosis	4	1	1	3		2	3	3	4	3	3	5		3	3	2	2.9	3	3	3	2	2.8	3	3	3.0	3	1	2	2.0	2.6	2				3	2	2.3	2.7
Ornithosis	4	3	2	3		3	2	2	3	3	3	3	3	1	3	3	2.7	4	2	2	3	2.8	3	2	2.5	1	1	2	1.7	2.2	2			3	2	3	2.5	2.5
Pasteurellosis	2	4	3	3		3	3	2	2	3	2	2	3	2	4	3	2.7	3	2	3	2	2.5	3	3	3.0	2	2	2	2.0	2.4	3			3	3	3	3.0	2.7
Colibacillosis	1	3	3	2		2	3	3	2	3	3	1		2	3	2	2.4	2	2	3	2	2.3	2	2	2.0	3	2	2	2.3	2.2	2			2	1	2	1.8	2.2
Mycoses	3	3	4	3		3	3	4	3	3	2	3	4	2	5	3	3.2	3	2	4	2	2.8	3	4	3.5	4	2	1	2.3	2.8	2			3	3	3	2.8	3.0
Mycotoxicoses	2	3	2	4		3		3	1	3	2	2	4	3	2	3	2.6	3	2	4	3	3.0	3	4	3.5	4	2	3	3.0	3.1	2			3	2	3	2.5	2.8
Mycoplasmosis	2	3	2	3		2	2	3	3	3	2	1		2	3	3	2.4	3	2	3	2	2.5	1	4	2.5	3	1	2	2.0	2.3	2			3	2	3	2.5	2.4
Fowl Pox	3	2	5	3		3		3	5	3	3	5		3		4	3.5	3	3		5	3.7	3	4	3.5	4	3	2	3.0	3.4	3				3	3	3.0	3.4
Adenoviruses (HE)	3	3		3		3	3	1	3	4	4	3		3	3	2	2.9	3	2	3	2	2.5	3	4	3.5	3	1	2	2.0	2.6	3			3	3	3	3.0	2.8
Inf. Bursal Disease	1	3		4		3	2	3	4	3				3	3	2	2.8	2	3	3		2.8	3	2	2.5	2	3	4	3.0	2.8	3				3	3	3.0	2.8
Influenza	4	3	2	3		2	3	2	3	3	3	3		3	3	2	2.8	2	2	3	1	2.0	1	2	1.5	1	2	2	1.7	1.8	2				1	2	1.7	2.3
Newcastle Disease	3	3	3	3		3	3	3	2	3	3			2	3	3	2.8	2	3	3	3	2.8	2	4	3.0	3	2	2	2.3	2.7	2				3	3	2.7	2.8
Oth. Neoplasms (RE,LPD)	4	3		3		3	3	3	3	3	3	3		3	3	3	3.1	3	3	4	2	3.0	3	3	3.0	3	2	2	2.3	2.8	3			3	3	3	3.0	3.0
Immunogenetics	3	1	2	3		3	3	3	1	2	3			2	1	3	2.3	2	3	3	3	2.8	3	3	3.0	3	1	3	2.3	2.7	3			3	3	2	2.8	2.5
$\bar{x}$	2.7	2.8	2.8	3.0		2.7	2.8	2.7	2.8	3.0	2.8	2.8	3.4	2.4	3.1	2.7	2.8	2.7	2.4	3.1	2.5	2.6	2.5	3.1	2.8	2.7	1.7	2.3	2.3	2.6	2.4			2.9	2.5	2.7	2.6	2.7

INTERPRETIVE COMMENTS

At various times during the review, there were discussions on the role of SEA-AR in poultry disease research. The following is a summary of some of the comments:

SEA-AR has areas of significant strengths that need to be kept strong. It should concentrate major programs at those locations where specialized facilities and personnel are located and not spend time on small scattered 1/2 SY efforts. Thus, the agency is encouraged to keep the programs strong and not to dilute them.

SEA-AR should have more enduring programs than the State Agricultural Experiment Stations. The programs should be concentrated on long range, complex, high priority problems. The State Agricultural Experiment Stations tend to be more fickle because of the uncertainty of funding. SEA-AR is in a unique position to conduct research in those areas not having immediate practical benefit. However, the Agency should be in a position to respond to some short-range and emergency problems, i.e., "You must solve short-range problems if you want to live long enough to solve long-range problems."

Cooperation between AR and CR and integration of research programs of AR and CR are of paramount importance.

In spite of its stable, long-term outlook, SEA-AR should also be able to respond to occasional field problems. The Agency must also be flexible enough to handle emergencies because the industry looks to SEA-AR and the SAES to handle emergencies.

SEA-AR research is recognized worldwide. As a group, the scientists are highly respected. In general, programs are balanced and on target.

CONCLUSIONS AND RECOMMENDATIONS

The scientists at all locations are to be commended for their productivity and for the quality of the research. Each group could benefit by having additional support and personnel to strengthen the programs. Technical support is the most important need.

Regional Poultry Research Laboratory, East Lansing, MI

1. Research on lymphoid leukosis is important and should not be decreased.

2. The redirection of laboratory effort from Marek's disease research into research on adenoviruses and infectious bursal disease is appropriate particularly as there is a relatively large effort in the states on Marek's Disease.

Poultry Protozoan Parasite Laboratory, Beltsville, MD

1. As has been recognized in the BARC review held on September 26, 1980, the program has been turned around since the poultry diseases program review in 1975. The research is on track. The enthusiasm of the group is commendable.

Southeast Poultry Research Laboratory, Athens, GA

1. A new scientist for research on salmonella should be hired as soon as possible.

2. The reduced effort on Mycoplasma synoviae at other locations may necessitate additional emphasis on this organism.

South Central Poultry Research Laboratory, Mississippi State, MS

1. There is a need for a professional position in the diseases area, however, the area of the specialty should be identified in order to recruit the right person. Research could be in the areas of mycoplasmosis, colibacillosis, or ethology.

2. The newly acquired feed pelleting facility offers an opportunity to study ways of eliminating salmonella from feed using pelleting.

National Animal Disease Center, Ames, IA

1. The ornithosis program is weak on animal applications. A veterinary medical officer should be hired for the project as soon as possible.

2. The new program on colibacillosis is off to a good start. Because of the importance of this disease in poultry, emphasis on it should be increased.

3. The program on mycosis and mycotoxicosis is well supported though another veterinarian can be used for animal applications.

4. The program on pasteurellosis is strong and exciting; it has a good balance in animal work; it is to be commended.

Constraints on Productivity

1. The major constraint is the shortage of technicians. Even though this is not possible with current ceilings, the goal should be to have a full-time technician for each scientist.

2. There are no significant constraints on buildings and equipment. Additional use could be made of the pelleting facilities at Mississippi State.

3. Start-up funds are insufficient to hire a veterinarian at Mississippi State and will limit research unless new funds are found.

Productivity

1. There are a few scientists whose productivity is below what is expected. The supervisors have been informed and will be taking remedial action.

Responses to Recommendations of the 1975 Review

1. Research on influenza is now being conducted at the Southeast Poultry Research Laboratory. Additional effort is needed.

2. Additional funding has been obtained for salmonelloses. Attempts are being made to hire an additional veterinarian for the research.

3. With a new leader, research on fowl cholera is progressing well.

4. With the retirement of the leader of the ornithosis research, this program has suffered a set back. Attempts are being made to recruit a replacement.

5. The parasitology program has been redirected to emphasize methods of control other than chemoprophylactic, and there is a closer association with industry and its problems.

6. Research on infectious bursal disease has been started at the Regional Poultry Research Laboratory.

7. Extramural support was given to research on reovirus infections. A vaccine has been developed and is ready for licensing.

8. Significant fine tuning occurred, i.e., minor redirections of individual research programs.

9. Representatives from the Southern Region have participated in the NE109 Technical Committee Meetings.

10. The National Poultry Improvement Plan has been transferred to APHIS.

Program Review of Poultry Parasitic Diseases Lab

A program review was held September 26, 1980, by Dr. Putnam. The recommendations of the laboratory chief and the scientists for the program are reinforced by this review. The major recommendations that (1) the laboratory explore every avenue for increased funding, and (2) the scientists make every effort to improve contacts with industry, are also supported.

Participation by Industry

1. Various industry groups have participated in the AR research program in many ways. They have cooperated in experiments. They have supported funding in Congress. Most importantly they have participated greatly in this program review. This industry support is greatly appreciated.